(54) Title: SYNTHETIC SINGLE DOMAIN POLYPEPTIDES MIMICKING APOLIPOPROTEIN E AND METHODS OF USE

Ac-R18L-NH$_2$ i.v. (100 µg/mouse)
Apo E null, fasted
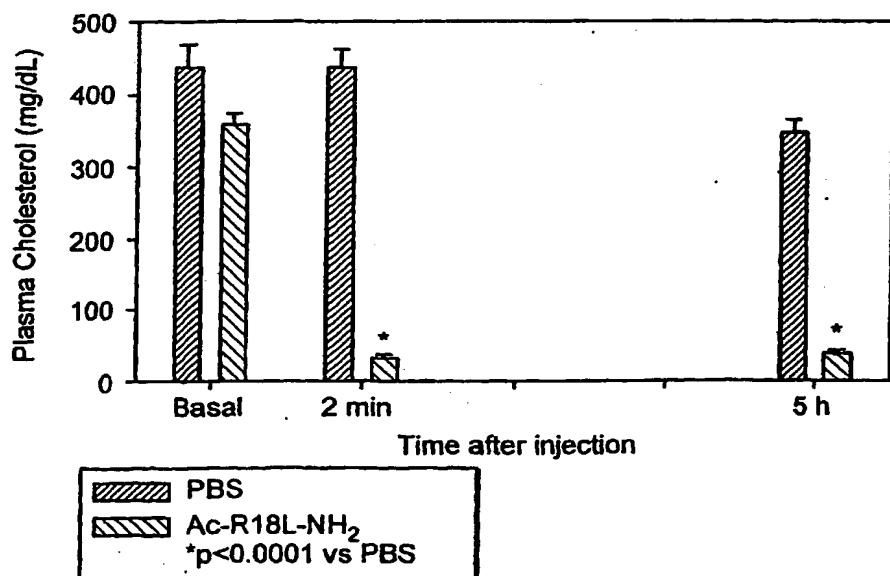
(57) Abstract: The present invention is directed to a synthetic apolipoprotein-E mimicking polypeptide consisting of a single domain. The invention is also directed to nucleic acid encoding the polypeptide, vectors including the nucleic acid, antibodies specific for the polypeptide, and compositions comprising the same and methods of using the same.

SYNTHETIC SINGLE DOMAIN POLYPEPTIDES MIMICKING APOLIPOPROTEIN E AND METHODS OF USE

FIELD OF THE INVENTION

5   This invention relates to the field of molecular biology and protein biology including polypeptides and polypeptide mimics. This application also relates to the field of cholesterol metabolism, catabolism and the treatment and management of cholesterol associated conditions.

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BACKGROUND OF THE INVENTION

20   Plasma lipoproteins and Coronary artery disease. Epidemiological studies indicate that increased plasma cholesterol levels increase the risk for atherosclerosis. Five completed major trials have provided conclusive evidence of a benefit from treatment aimed primarily at reducing low-density lipoprotein (LDL) -cholesterol (Illingworth R.D., et al. *Current Opini. Lipidol.*
25   1999, 10:383-386). Among other lipoprotein risk factors is familial dysbetalipoproteinemia, which results in the accumulation of remnant atherogenic lipoproteins derived from the catabolism of chylomicron and VLDL (Kwiterovich, P.O., Jr. *Am. J. Cardiol.* 1998, 82:3U-7U). It has been shown that a 1% decrease in the plasma cholesterol level decreases the risk of

coronary artery disease by 2% (Deedwania, P.C. *Med. Clin. North Am.* 1995, 79:973-998). The focus of angiographic trials has been on LDL reduction and these studies have demonstrated that decreases of LDL-cholesterol of more than 30% to 35% are associated with lower rates of coronary events (Watts,

5   G.W., et al. *Atherosclerosis* 1998, 414:17-30). There is also growing evidence that triglyceride-rich lipoproteins may adversely affect endothelial function and increase oxidative stress by promoting the production of small, dense LDL and by reducing high-density lipoprotein (HDL) levels (Marais, D., *Curr. Opin. Lipidol.* 2000, 11:597-602).

10 Anti-atherogenic properties of Apolipoprotein E (Apo E).
Apolipoprotein E is a protein that binds lipid and has two major domains (Mahley, R.W., et al. *J. Lipid Res.* 1999, 40:622-630). The 22 kDa amino terminal domain has been shown by X-ray crystallographic studies to be a 4-helix bundle (Wilson, C., et al. *Science* 1991;252:1817-1822) and to contain a

15 positively-charged receptor binding domain. For this region to mediate very low-density lipoprotein (VLDL) binding to its receptors, the apolipoprotein must associate with the lipoprotein surface; this is enabled by the C-terminal amphipathic helical region. If the 4-helix bundle that contains the positively charged receptor-binding domain does not open up on the lipoprotein

20 surface, then the VLDL is defective in binding to receptors. Thus, the positively charged arginine (Arg)-rich cluster domain of the Apo E and the C-terminal amphipathic helical domain, are both required for the enhanced uptake of atherogenic Apo E-containing lipoproteins.

Chylomicron is a lipoprotein found in blood plasma, which carries

25 lipids from the intestines into other body tissues and is made up of a drop of triacylglycerols surrounded by a protein-phospholipid coating. Chylomicron remnants are taken up by the liver (Havel, R.J., 1985, *Arteriosclerosis.* 5:569-580) after sequestration in the space of Disse, which is enriched with Apo E

(Kwiterovich, P.O., Jr., 1998; Deedwania, P.C., 1995; and Watts, G.W., et al.,
1998). Apo E is the major mediator of hepatic remnant lipoprotein uptake by
the LDL receptor or LRP. Lipolysis of normal VLDL Sf (subfraction) of more
than 60 permit binding of the lipolytic remnant to the LDL receptor

5   (Catapano, A.L. et al. 1979, *J. Biol. Chem.* 254:1007-1009; Schonfield, G., et al.
1979. *J. Clin. Invest.* 64:1288-1297). Lipoprotein lipase (LpL) may facilitate
uptake through localization of Apo B-containing lipoproteins to membrane
heparan sulphate proteoglycan (HSPG) (Eisenberg, et al. 1992. *J. Clin. Invest.*
90:2013-2021; Hussain, M., et al., *J. Biol. Chem.* 2000, 275:29324-29330) and/or

10   through binding to the LDL-receptor-related protein (LRP) (Beisiegel, U., et
al., 1989, *Nature* 341:162-164). Cell-surface HSPG may also function as a
receptor and has variable binding affinities for specific isoforms of Apo E. In
particular, Apo E is synthesized by the liver and also by
monocyte/macrophages, where it exerts its effect on cholesterol homeostasis.

15   *In vivo* evidence for the local effect of lack of Apo E comes from the
observations of Linton and Fazio, who showed accelerated atherosclerosis in
C57BL/6 mice transplanted with bone marrow from Apo E-deficient mice
(Linton, M.F. and Fazio, S. *Curr. Openi. Lipidol.* 1999, 10:97-105). Apo E-
dependent LDL cholesteryl ester uptake pathway has been demonstrated in

20   murine adrenocortical cells (Swarnakar, S., et al. *J. Biol. Chem.* 2001, 276:21121-
21126). This appears to involve chondroitin sulphate proteoglycan (CSPG)
and a 2-macroglobulin receptor.

      It has been shown that the receptor-binding domain of Apo E, rich in
Arg residues (141-150), covalently linked to a synthetic class A amphipathic-

25   helical domain, enhances the hepatic atherogenic lipoprotein uptake (Datta, G.
et al. *Biochemistry* 2000, 30:213-220). Recent studies indicate that a potential
anti-atherogenic action of Apo E is that it stimulates endothelial production of
heparan sulfate (HS) (Paka, L., et al. *J. Biol. Chem.* 1999, 274:4816-4823).

Lipoproteins are complexes of one or more lipids bound to one or more proteins and transport water-insoluble fats in the blood. Cholesterol is carried through the bloodstream by lipoproteins. There are no agents available which reduce cholesterol via the binding mechanisms of lipoproteins. There is a

5   need for more effective agents that are capable of reducing cholesterol in a subject so as to reduce diseases and conditions which are associated with increased cholesterol.

SUMMARY OF THE INVENTION

10   The present invention provides polypeptides, compositions and methods for increasing uptake of cholesterol in a subject.

In one aspect, the invention is directed to a synthetic apolipoprotein-E mimicking polypeptide comprising consecutive amino acids having an amino acid sequence selected from the group of (i) X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-

15   Y-Arg-X-Y-Y-X or the reverse sequence thereof, (ii) Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y or the reverse sequence thereof, (iii) Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y-Y-X or the reverse sequence thereof, and (iv) X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg or the reverse sequence thereof, wherein X is glycine, threonine, serine or alanine, wherein Y is a hydrophobic amino acid, wherein

20   the polypeptide comprises an acetyl group at the N-terminus and an amide group at the C-terminus, and wherein the polypeptide consists of a single domain.

In one embodiment of the polypeptide of the invention, Y is selected from the group consisting of phenylalanine, tyrosine, leucine, isoleucine,

25   valine, and tryptophan. In another embodiment, the polypeptide comprises from about 10 amino acids to about 30 amino acids in length. In other embodiments, the invention provides a polypeptide comprising a sequence of consecutive amino acids selected from the group consisting of SEQ ID NOS:1-

207. The invention also provides a specific embodiment wherein the polypeptide comprises the sequence Gly-Ile-Arg-Arg-Phe-Leu-Gly-Ser-Ile-Trp-Arg-Phe-Ile-Arg-Ala-Phe-Tyr-Gly (SEQ ID NO:5). In other embodiments, the polypeptide comprises a recombinant polypeptide, a synthetic

5   polypeptide, and/or a peptidomimetic.

The present invention also provides peptides, which are capable of reducing cholesterol in subjects. In other aspects, the invention is also directed to compositions including the polypeptide and methods of using the polypeptide to reduce serum cholesterol in a subject.

10   In a specific aspect, the invention provides a composition comprising a nucleic acid encoding a peptide of the invention. In another aspect, the invention provides a composition comprising a peptide of the invention, wherein the composition is a protein. In one embodiment, the composition comprises a peptide which is encoded by a nucleic acid. In another

15   embodiment, the nucleic acid is encoded in a vector. In a specific embodiment, the composition further comprises a pharmaceutically acceptable carrier. In another embodiment, the composition comprises an adjuvant.

The invention also provides, in another aspect, methods of increasing

20   the effectiveness of the composition comprising a peptide of the invention, by combining the composition with an adjuvant.

In still another aspect, the invention provides an isolated nucleic acid encoding the polypeptide of the invention. In some embodiments, the nucleic acid comprises DNA, RNA and/or cDNA. In another aspect, the invention

25   provides a vector comprising the nucleic acid. The invention also provides a host cell comprising the nucleic acid of the invention. In one embodiment, the cell is eukaryotic or prokaryotic.

Additionally, the invention provides a peptide that enhances low-density lipoprotein (LDL) binding and very low-density lipoprotein (VLDL) binding to a cell and/or that enhances low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) degradation by a cell.

5   In another aspect, the invention provides a composition comprising a polypeptide according to the invention and a pharmaceutically acceptable carrier. In a specific embodiment, the carrier comprises dimyristoylphosphatidyl (DMPC), phosphate buffered saline, a time release formulation or a multivesicular liposome.

10   In another aspect, the invention provides methods of increasing the solubility of the composition of the invention, comprising combining the composition with a solubilizing agent.

In yet another aspect, the invention provides a monoclonal antibody that specifically binds to the Apo E-derived polypeptide of the invention.

15   In still another aspect, the invention provides a composition including a recombinant cell expressing the nucleic acid encoding the peptide of the invention and a carrier. In addition, the invention provides a composition comprising a recombinant cell producing the polypeptide and a carrier.

The invention also provides a transgenic non-human subject expressing

20   the nucleic acid according to the invention. In one embodiment, the subject is an animal or a plant. In another embodiment, the transgenic non-human subject synthesizes and/or produces the polypeptide.

The invention provides methods for using the peptides of the invention. In one aspect, the invention provides a method for enhancing LDL

25   binding to and/or uptake by a cell, the method comprising mixing, contacting and/or associating the cell with the polypeptide of the invention, thereby allowing the polypeptide to bind the LDL and enhance LDL binding and/or uptake with the associated cell. In another aspect, the invention provides a

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method for enhancing LDL binding and VLDL binding to a cell in a subject. The method comprises administering the polypeptide, or a composition thereof, to the subject in an amount effective to increase LDL and VLDL binding to the cell of the subject.

5   Also provided are methods of reducing serum cholesterol in a patient. In one aspect, the method comprises administering to the patient a composition comprising a peptide of the invention, wherein the peptide enhances cellular uptake of cholesterol in the patient, thereby reducing the patient's serum cholesterol. In another aspect, the invention provides a

10   method for reducing serum cholesterol in a subject, the method comprising the step of administering to the subject an amount of the polypeptide or a composition thereof, effective to increase binding of LDL and/or VLDL to cells in the subject, thereby reducing serum cholesterol in the subject.

In still another aspect, the invention provides methods of enhancing

15   LDL uptake in a patient, comprising administering to the patient a composition comprising a peptide of the invention, wherein the composition enhances cellular uptake of LDL in the patient and thereby enhances LDL uptake in the patient.

The invention also provides methods of enhancing VLDL uptake in a

20   patient, comprising administering to the patient a composition comprising a peptide of the invention, wherein the composition enhances cellular uptake of VLDL in the patient and thereby enhances VLDL uptake in the patient.

In another aspect, the invention provides for a method of treating a subject with a disease or condition associated with high serum cholesterol,

25   comprising administering to the subject an amount of the polypeptide of the invention effective to reduce serum cholesterol, thereby ameliorating the disease or condition.

In yet another aspect, the invention provides for a method for treating a subject with coronary artery disease, the method comprising the step of administering to the subject an amount of the polypeptide, or a composition thereof, effective to increase cellular uptake of serum cholesterol in the subject

5   to thereby treat the subject. In one embodiment, the disease or condition associated with high serum cholesterol is dysbetalipoproteinemia.

In still another aspect, the invention provides a method for reducing the risk of myocardial infarction in a subject, the method comprising the step of administering to the subject an amount of the polypeptide, or a

10   composition thereof, effective to increase cellular uptake of serum cholesterol in the subject, to thereby reduce the risk of myocardial infarction in the subject.

In another aspect, the invention provides a method of reducing a blockage in the circulatory system of a patient comprising administering to

15   the patient a composition comprising the polypeptide of the invention in an amount effective to increase uptake of cholesterol in the subject so as to reduce the blockage in the circulatory system of the patient. In one embodiment, the composition is administered directly to the location of the blockage in the subject. In another embodiment, the composition is administered to a patient

20   in combination with a thrombolytic agent, such as aspirin.

The invention also provides methods of breaking an embolus in a patient comprising administering to the patient a composition comprising a peptide of the invention in an amount effective to increase uptake of cholesterol in the subject so as to break an embolus in the patient.

25   In another aspect, the invention provides for a method for treating atherosclerosis in a subject, the method comprising the step of administering the to subject an amount of a polypeptide or a composition of the invention effective to bind serum cholesterol and/or enhance cellular uptake of serum

cholesterol in the subject, thereby treating atherosclerosis in the subject. In addition, the invention provides a method for reducing plaque formation on vessel walls.

In yet another embodiment, the invention provides methods of

5   reducing the risk of a stroke in a patient comprising administering to the patient a composition comprising a peptide of the invention in an amount effective to increase cellular uptake of cholesterol in the patient and thereby reduce the risk of stroke in the patient.

In addition, the invention provides methods of reducing the risk of

10   myocardial infarction in a patient comprising administering to the patient the composition comprising a peptide of the invention in an amount effective to increase cellular uptake of cholesterol in the patient and thereby reduce the risk of myocardial infarction in the patient.

In yet another aspect, the invention provides for use of the polypeptide

15   for the making of a composition to treat a disease associated with increased serum cholesterol in a subject. In still another aspect, the invention provides for use of the polypeptide for the making of a composition to reduce LDL and/or VLDL serum levels in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

5 These are non-limiting examples.

Figures 1A-1F are representations of space-filling molecular models of the 18L peptide, the R-18L peptide and the 18A peptide developed to study the cross-sectional shape of these molecules. Space filling models (Figs. 1D, 1E and 1F) are shown along with the approximate cross sectional shape (Figs.

10 1A, 1B and 1C). The cross sectional molecular shapes presented here show the cross-sectional area of the head groups versus the fatty acyl chains of the synthetic peptide analogs of the invention.

Figure 2 is a graphic representation showing the effect of the 18A peptide, the 18L peptide, the R-18L peptide and the dimethyl-lysine (DiMeK)

15 18L polypeptides on red blood cells as measured by percent hemolysis.

Figure 3A is a graphic representation showing the uptake by HepG2 cells of ^{125}I-labelled VLDL in the presence of R-18L.

Figure 3B is a graphic representation showing the uptake by HepG2 cells of ^{125}I-labelled VLDL in the presence of R-18L.

20 Figure 4 is a graphic representation showing reduction of plasma cholesterol in blood samples from fasted apo E null mice after injection with peptide Ac-(R)18L-NH$_2$ compared with plasma cholesterol levels taken immediately before injection (basal levels) with the peptide. Plasma cholesterol levels were measured at 2 min after injection and 5 hours after

25 injection. Control apo E null animals were injected with PBS.

Figure 5 is a graphic representation showing dose dependency of cholesterol reduction in blood samples from apo E null mice taken at zero (0)

and five (5) hours after intravenous injection with peptide Ac-(R)18 L-NH₂ at

10 µg, 25 µg or 50 µg doses. The data are expressed as mean ± SEM.

Figure 6 is a graphic representation showing the mg of total fecal

cholesterol in R18L peptide-injected animals versus control animals.

5   Figure 7 is a graphic representation showing the plasma lipoprotein

profile from an *in vivo* mixing experiment. The relative cholesterol eluting

shows three peaks: VLDL, LDL and HDL at elution volume (mL) c. 12, 16 and

18. The counts per minute (CPM) detected show peaks at similar intensity

and at the same elution volume as the cholesterol. VLDL shows a CPM of c.

10   50, LDL shows a CPM of c. 150 and HDL shows a CPM of c. 350.

Figure 8A is a representation of a helical wheel molecular model of R

14 L-1 peptide derived from peptide R 18L. The letters are abbreviations for

amino acids. The arginines are shown to carry a positive charge.

Figure 8B is a representation of a helical wheel molecular model of R 14

15   L-2 peptide derived from peptide R 18L. The letters are abbreviations for

amino acids. The arginines are shown to carry a positive charge. The bold

circles around the letter abbreviations indicate hydrophobic residues.

Figure 9 is a graphic representation showing the effect of R14L

peptides (R14 L containing 2 Ile residues) and R14 L (containing 1 Ile residue)

20   on the uptake of human LDL in CHO cells measured by uptake of ¹²⁵I-LDL in

ng of Apo B/mg cell protein.

Figure 10 is a graphic representation showing basal cholesterol levels of

apo E null mice injected with two different single domain cationic peptides

(Ac-(R)14L-NH₂ and Ac-(R)18L-NH₂) two (2) minutes and five (5) hours after

25   the time of injection.

Figure 11 is a graphic representation showing basal cholesterol levels of

apo E null mice injected with Ac-(R)14L-NH₂ two (2) minutes or five (5) hours

after injection with free peptide Ac-(R)14L-NH₂ at pH 7.4, with peptide:DMPC

complex at pH 5.0 (1:1 w/w) or with peptide:DMPC complex at pH 7.4 (1:1 w/w).

Figures 12A-12F are graphical illustrations of detergent micelles, membrane bilayers and inverted bilayers.

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DETAILED DESCRIPTION OF THE INVENTION

All patents, patent applications and publications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entireties into this application in order to more fully describe the state of the art as known to

10   those skilled therein as of the date of the invention described and claimed herein.

It is to be understood that this invention is not limited to specific synthetic methods, or to specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, to

15   specific pharmaceutical carriers, or to particular pharmaceutical formulations or administration regimens, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

20   Definitions and Nomenclature

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" can include plural referents unless the context

25   clearly dictates otherwise. Thus, for example, reference to "a compound" includes mixtures of compounds, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range

5   by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as

10   approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

The amino acid abbreviations used herein are conventional one letter

15   codes for the amino acids and are expressed as follows: A, alanine; B, asparagine or aspartic acid; C, cysteine; D aspartic acid; E, glutamate, glutamic acid; F, phenylalanine; G, glycine; H histidine; I isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; Z, glutamine or

20   glutamic acid.

"Polypeptide" as used herein refers to any peptide, oligopeptide, polypeptide, gene product, expression product, or protein. A polypeptide is comprised of consecutive amino acids. The term "polypeptide" encompasses naturally occurring or synthetic molecules.

25   In addition, as used herein, the term "polypeptide" refers to amino acids joined to each other by peptide bonds or modified peptide bonds, *e.g.*, peptide isosteres, etc. and may contain modified amino acids other than the 20 gene-encoded amino acids. The polypeptides can be modified by either

natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. The same type of

5 modification can be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide can have many types of modifications. Modifications include, without limitation, acetylation, acylation, ADP-ribosylation, amidation, covalent cross-linking or cyclization, covalent attachment of flavin, covalent attachment of a heme moiety, covalent

10 attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphytidylinositol, disulfide bond formation, demethylation, formation of cysteine or pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation,

15 pergylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. (*See Proteins – Structure and Molecular Properties* 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); *Posttranslational Covalent Modification of Proteins*, B.C.

20 Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)).

As used herein, the term "amino acid sequence" refers to a list of abbreviations, letters, characters or words representing amino acid residues.

As used herein, "peptidomimetic" means a mimetic of a peptide which includes some alteration of the normal peptide chemistry. Peptidomimetics

25 typically enhance some property of the original peptide, such as increase stability, increased efficacy, enhanced delivery, increased half life, etc. Methods of making peptidomimetics based upon a known polypeptide sequence is described, for example, in U.S. Patent Nos. 5,631,280; 5,612,895;

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and 5,579,250. Use of peptidomimetics can involve the incorporation of a non-amino acid residue with non-amide linkages at a given position. One embodiment of the present invention is a peptidomimetic wherein the compound has a bond, a peptide backbone or an amino acid component

5   replaced with a suitable mimic. Some non-limiting examples of unnatural amino acids which may be suitable amino acid mimics include β-alanine, L-α-amino butyric acid, L-γ-amino butyric acid, L-α-amino isobutyric acid, L-ε-amino caproic acid, 7-amino heptanoic acid, L-aspartic acid, L-glutamic acid, N-ε-Boc-N-α-CBZ-L-lysine, N-ε-Boc-N-α-Fmoc-L-lysine, L-methionine

10   sulfone, L-norleucine, L-norvaline, N-α-Boc-N-δCBZ-L-ornithine, N-δ-Boc-N-α-CBZ-L-ornithine, Boc-p-nitro-L-phenylalanine, Boc-hydroxyproline, and Boc-L-thioproline.

The word "or" as used herein means any one member of a particular list and also includes any combination of members of that list.

15   The phrase "nucleic acid" as used herein refers to a naturally occurring or synthetic oligonucleotide or polynucleotide, whether DNA or RNA or DNA-RNA hybrid, single-stranded or double-stranded, sense or antisense, which is capable of hybridization to a complementary nucleic acid by Watson-Crick base-pairing. Nucleic acids of the invention can also include nucleotide

20   analogs (e.g., BrdU), and non-phosphodiester internucleoside linkages (e.g., peptide nucleic acid (PNA) or thiodiester linkages). In particular, nucleic acids can include, without limitation, DNA, RNA, cDNA, gDNA, ssDNA, dsDNA or any combination thereof

As used herein, "reverse analog" or "reverse sequence" refers to a

25   peptide having the reverse amino acid sequence as another, reference, peptide. For example, if one peptide has the amino acid sequence ABCDE, its reverse analog or a peptide having its reverse sequence is as follows: EDCBA.

Compounds and Compositions of the Invention

The present invention is directed to a synthetic apolipoprotein-E mimicking peptide or polypeptide. The polypeptide may comprise an amino acid sequence selected from the group of (i) X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y-Y-X, or the reverse sequence thereof, (ii) Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y, or the reverse sequence thereof, (iii) Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y-Y-X, or the reverse sequence thereof, and (iv) X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg, or the reverse sequence thereof, where X is glycine, threonine, serine or alanine, where Y is a hydrophobic amino acid, where the polypeptide comprises an acetyl group at the N-terminus and an amide group at the C-terminus, and where the polypeptide consists of a single domain.

In one embodiment, Y is selected from the group consisting of phenylalanine, tyrosine, leucine, isoleucine, valine, tryptophan, and combinations thereof. In another embodiment, the polypeptide comprises from about 10 amino acids to about 30 amino acids in length. In a further embodiment, the peptide of the invention comprises from about 14 amino acids to about 18 amino acids. The peptide of the invention can be about 14 amino acids in length or about 18 amino acids in length. In a further embodiment, the polypeptide comprises a sequence of consecutive amino acids selected from the group of SEQ ID NOS:1-207. In another embodiment, the polypeptide comprises the sequence Gly-Ile-Arg-Arg-Phe-Leu-Gly-Ser-Ile-Trp-Arg-Phe-Ile-Arg-Ala-Phe-Tyr-Gly (SEQ ID NO:5). In a further embodiment, the polypeptide comprises a recombinant polypeptide. In another embodiment, the polypeptide comprises a synthetic polypeptide. In another embodiment, the polypeptide comprises a peptidomimetic.

The invention also provides a polypeptide that enhances low-density lipoprotein (LDL) - and very low density lipoprotein (VLDL) - binding to a cell. In one embodiment, the polypeptide enhances low-density lipoprotein

(LDL)- and very low density lipoprotein (VLDL)- degradation by a cell. In addition, the invention provides a mixture of one or more of the peptides described herein, wherein the mixture of peptides is administered to a subject to reduce cholesterol in the subject.

5

Non-limiting Examples of Polypeptides and Peptides of the Invention

Non-limiting examples of peptides of the invention are given below. A number of possible analogs of class L peptides ranging from about 18 amino acids to about 10 amino acids in length were synthesized using art-recognized

10   methods. The peptides are protected at the N-terminus by an acetyl group and at the C-terminus by an amide group. Table 1 shows non-limiting examples of peptides that are analogs or reverse analogs of the 18L peptide which have an increased angle subtended by its helix.

15

Table 1

Amino Acid Sequence	SEQ ID NO:
GIRRFLGSIWRFIRAFYG-	13
GIWRFLGSIRRFIRAFYG	14
GIGRFLRSIWGFIRAFYR	15
GIRRFLGSIWRFIGAFYR	16
GIRRFLGSIWAFIRRFYG	17
GIRRFLSGIWRFIRAFYG	18
GIRRFLSGIWAFIRAFYG	19
GIWRFLSGIRRFIRAFYG	20
GIRRFLGAIWRFIRSFYG	21
GIWRFLGAIWRFIRSFYG	22
Reverse analogs (reverse amino acid sequence of the above peptides)	
GYFARIFRWISGLFRRIG	23
GYFARIFRRISGLFRWIG	24

RYFARIFGWISRLFRGIG	25
RYFAGIFRWISRLFRGIG	26
GYFRRIFAWISGLFRRIG	27
GYFARIFRWIGSLFRRIG	28
GYFARIFRWIGSLFRRIG	29
GYFRRIFRRIGSLFAWIG	30
GYFSRIFRWIAGLFRRIG	31
GYFSRIFRWIAGLFRWIG	32

Table 2 shows non-limiting examples of DiMet-lysine analogs and reverse analogs of peptides.

Table 2

Amino Acid Sequence of Analogs, wherein K'=(DiMe)Lys	SEQ ID NO:
GIK'K'FLGWIK'AFISK'FYG	33
GIWK.FLGSIK'K'FIK'AFYG	34
GIGK'FLK'SIWGFIK'AFYK'	35
GIK'K'FLGSIWK'FIGAFYK'	36
GIK'K'FLGSIWAFIK'K'FYG	37
GIK'K'FLSGIWK'FIK'AFYG	38
GIK'K'FLSGIWFIAK'K'FYG	39
GIWK'FLSGIK'K'FIK'AFYG	40
GIK'K'FLGAIWK'FIK'SFYG	41
GIWK'FLGAIK'K'FIK'SFYG	42
Reverse analogs	
GYFAK'IFK'WISGLFK'K'IG	43
GYFAK'IFK'K'ISGLFK'WIG	44
K'YFAK'IFGWISK'LFK'GIG	45
K'YFAGIFK'WISK'LFK'GIG	46
GYFK'K'IFAWISGLFK'K'IG	47
GYFAK'IFK'WIGSLFK'K'IG	48
GYFAK'IFK'WIGSLFK'K'IG	49
GYFK'K'IFK'K'IGSLFAWIG	50
GYFSK'IFK'WIAGLFK'K'IG	51
GYFSK'IFK'K'IAGLFK'WIG	52

5

Table 3 shows non-limiting examples of polypeptides of the invention having enhanced solubility.

Table 3

Amino Acid Sequence	SEQ ID NO:
GIK'RFLGSIWRFIK'AFYG	53
GIWK'FLGSIRRFIK'AFYG	54
GIGK'FLRSIWGFIRAFYK'	55
GIK'RFLGSIWRFIGAFYK'	56
GIRK'FLGSIWAFIK'RFYG	57
GIRK'FLSGIWRFIK'AFYG	58
GIRK'FLSGIWAFIK'AFYG	59
GIWK'FLSGIRRFIK'AFYG	60
GIK'RFLGAIWRFIK'SFYG	61
GIWK'FLGAIWRFIK'SFYG	62
Reverse analogs	
GIK'RFLGWIK'AFISRFYG	63
GYFAK'IFRWISGLFK'RIG	64
GYFAK'IFRRISGLFK'WIG	65
RYFAK'IFGWISRLFK'GIG	66
RYFAGIFK'WISRLFK'GIG	67
GYFRK'IFAWISGLFK'RIG	68
GYFAK'IFRWIGSLFK'RIG	69
GYFAK'IFRWIGSLFRK'IG	70
GYFK'RIFRK'IGSLFAWIG	71
GYFSK'IFRWIAGLFK'RIG	72
GYFSRIFRWIAGLFRWIG	73

In one embodiment, the amino acid sequence of a polypeptide of the invention is varied with regard to the ratio of arginine compared to dimethyl-lysine. For example, a peptide is synthesized with one to three arginine residues per peptide. Table 4 shows non-limiting examples of such analogs and reverse analogs.

Table 4

Amino acid sequence	SEQ ID NO:	Reverse analogs	SEQ ID NO.
GIRKFLGSIWRFIKAFYG (2Lys and 2Arg analog of 18L)	74	GYFARIFKWISGLFRKIG	83
GIWKFLGSIRRFIKAFYG	75	GYFARIFKRISGLFKWIG	84
GIGRFLKSIWGFIRAFYK	76	RYFAKIFGWISKLFRGIG	85
GIRKFLGSIWRFIGAFYK	77	RYFAGIFRWISRLFRGIG	86
GIRKFLGSIWAFIRKFYG	78	GYFKRIFAWISGLFKRIG	87
GIRKFLSGIWRFIKAFYG	79	GYFAKIFRWIGSLFKRIG	88
GIRKFLSGIWRFIKAFYG	80	GYFAKIFRWIGSLFKRIG	89
GIWKFLSGIRRFIKAFYG	81	GYFKRIFRKIGSLFAWIG	90
GIRKFLGAIWRFIKSFYG	82	GYFARIFRWISGLFKRIG	101
GIRKFLGSIWRFIRAFYG (1Lys and 3Arg analog of 18L)	91	GYFARIFRKISGLFRWIG	102
GIWRFLGSIKRFIRAFYG	92	RYFARIFGWISKLFRGIG	103
GIGRFLKSIWGFIRAFYR	93	KYFAGIFRWISRLFRGIG	104
GIRRFLGSIWKFIGAFYR	94	GYFKRIFAWISGLFRRIG	105
GIRRFLGSIWAFIKRFYG	95	GYFAKIFRWIGSLFRRIG	106
GIRRFLSGIWRFIKAFYG	96	GYFAKIFRWIGSLFRRIG	107
GIRKFLSGIWAFIRAFYG	97	GYFRKIFRRIGSLFAWIG	108
GIWRFLSGIKRFIRAFYG	98	GYFSKIFRWIAGLFRRIG	109
GIRKFLGAIWRFIRSFYG	99	GIRRILGSFWRFFRAIYG	110
GIWRFLGAIWKFIRSFYG	100		

Table 5 shows non-limiting examples of peptides of the invention, the analogs

having aromatic amino acids substituted with hydrophobic amino acids.

5

Table 5

Amino Acid Sequence	SEQ ID NO:
GFRRILGSFWRIFRAIYG	111
GFRRILGSIWRFIRAFYG	112
GIRRFLGSIWRIFRAFYG	113
GIRRFLGSFWRIIRAFYG	114
GLRRFIGSIWRFIRAFYG	115
GLRRFIGSIWRFIRAFYG	116
GIRRFIGSIWRFLRAFYG	117
GIRRFLGSFWRIFRAIYG	118
GFRRFLGSFWRIIRAIYG	119
GIRRFLGSIYRFIRAFWG	120
GIRRFYGSIWRFIRAFLG	121
GYIARFIRWFSGLIRRFG	122
GYGARIFRWISGLIRRFG	123
GYFARFIRWISGLFRRIG	124
GYFARIFRWISGIFRRLG	125
GYIARIFRWFSGLFRRIG	126

Table 6 shows non-limiting examples of peptides of the invention with enhanced helicity. In these examples, Ile residues are replaced by Leu residues (helix promoting) to enhance helicity. In particular, the Ile pushes the alkyl chain apart in the helix structure due to branching at C-, in contrast to Leu side chain.

Table 6

Amino Acid Sequence	SEQ ID NO:
GLRRFIGSLWRFLRAFYG	127
GYFARLFRWLSGIFRRLG	128
GIRRFLGSLWRFLRAFYG	129
GYFARLFRWLSFLFRRIG	130
GLRRFLGSIWRFLRAFYG	131
GYFARLFRWISGLFRRLG	132

Table 7 shows non-limiting examples of peptides of the invention having a shorter length relative to other peptides of the invention having their N-terminal 2 to 4 amino acids deleted and C-terminal 2 to 4 amino acids deleted.

Table 7

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
GIRRFYGSIWRFIR	133	RFIRWISGYFRRIG	151
RIFRWISGYFRRIG	134	RIFRWISGYIRRFG	152
RRFYGSIWRFIRAF	135	RIFRWISGYFRRLG	153
FARIFRWISGYFRR	136	RLFRWISGYFRRIG	154
GLRRFYGSLWRFLR	137	GIRRFYGSIWRFLR	155
RLFRWLSGYFRRLG	138	GLRRFYGSIWRFIR	156
RRFYGSLWRFLRAF	139	GIRRFYGSLWRFIR	157
FARLFRWLSGYFRR	140	GIRRYFGSIWRFIR	158
GIRRFYGSIWRFLR	141	GIRRYFGSIWRFLR	159
RLFRWISGYFRRIG	142	GIRRYFGSLWRFIR	160
RRFYGSIWRFLRAF	143	RIFRWISGFYRRIG	161
FARLFRWISGYFRR	144	RLFRWISGFYRRLG	162
GIRRFYGSLWRFLR	145	RIFRWLSGFYRRIG	163
RLFRWISGYFRRLG	146	RLFRWLSGFYRRIG	164
RRFYGSIWRFLRAF	147	RFLRWISGYFRRIG	165
FARIFRWLSGYFRR	148	RFLRWISGFYRRIG	166
GFRRIYGSIWRFIR	149	GFRRLYGSIWRFIR	167
GIRRFYGSIWRIFR	150	GIRRFYGSIWRIFR	168

5

Table 8 shows non-limiting examples of peptides of the invention with varying Lysine (K) to Arginine (R) residue ratio: 3R and 1K.

Table 8

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
GIKRFYGSIWRFIR	169	RFIRWISGYFRKIG	187
RIFRWISGYFRKIG	170	RIFKWISGYIRRFG	188
RKFYGSIWRFIRAF	171	RIFRWISGYFRKLG	189
FARIFRWISGYFKR	172	KLFRWISGYFRRIG	190
GLKRFYGSLWRFLR	173	GIRRFYGSIWKFLR	191
RLFRWLSGYFRKLG	174	GLKRFYGSIWRFIR	192
RKFYGSLWRFLRAF	175	GIRRFYGSLWKFIR	193
FARLFRWLSGYFKR	176	GIRRYFGSLWRFIR	194
GIKRFYGSIWRFLR	177	GLRRYFGSIWRFLR	195
RLFRWISGYFRKIG	178	GIRRYFSGLWRFIR	196
RKFYGSIWRFLRAF	179	RFLRWISGFYRRIG	197
FARLFRWISGYFKR	180	RLFRWISGFYRRLG	198
GIRKFYGSLWRFLR	181	RFLRWLSGFYRRIG	199
RLFKWISGYFRRLG	182	RLIRWLSGFYRRFG	200
RRFYGSIWRFLKAF	183	RFLRWFSGYIRRIG	201
FAKIFRWLSGYFRR	184	RFLRWISGYFRRIG	202
GFRRIYGSIWRFIK	185	GFRRLYSGIWRFIR	203
GIRKFYGSIWRIFR	186	GIRRYFGSIWRIFR	204

Further non-limiting examples of the peptides of the invention include

peptides with an increased angle subtended, such as

GIRRFLGWIRAFISRFVG-Arg (analog of 18L) (SEQ ID NO:205); a peptide

with an increased angle DiMe-K — GIK′K′FLGWIK′AFISK′FVG, wherein

K′=DiMe-K (SEQ ID NO:206); and peptides with an enhanced solubility, such

as GIK′RFLGWIK′AFISRFVG (SEQ ID NO:207).

Numerous variants or derivatives of the peptides and analogs of the

invention are also contemplated. Non-limiting examples of the peptides and

analogs thereof have been described herein (see Tables 1-8, for example). As

used herein, the term "analog" is used interchangeably with "variant" and "derivative." Variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. Such, amino acid sequence modifications typically fall into one or more of three classes:

5   substantial; insertional; or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily are smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. These variants ordinarily are prepared by site-specific

10   mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid

15   substitutions are typically of single residues, but can occur at a number of different locations at once. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final derivative or analog. Substututional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions

20   generally are made in accordance with Table 9 and are referred to as conservative substitutions.

Table 9 – Amino Acid Substitutions

Original Residue	Non-limiting Exemplary Conservative Substitutions
Ala	Ser
Arg	Gly, Gln
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn, Lys
Glu	Asp
Gly	Ala
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substantial changes in function or immunological identity are made by

selecting substitutions that are less conservative than those in Table 9, *i.e.*,

selecting residues that differ more significantly in their effect on maintaining

(a) the structure of the polypeptide backbone in the area of the substitution,

for example as a sheet or helical conformation, (b) the charge or

hydrophobicity of the molecule at the target site, or (c) the bulk of the side

chain. The substitutions which in general are expected to produce the greatest

changes in the protein properties are those in which: (a) the hydrophilic

residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue,

e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is

substituted for (or by) any other residue; (c) a residue having an electropositive side chain, *e.g.,* lysyl, arginyl, or hystidyl, is substituted for (or by) an electronegative residue, *e.g.* glutamyl or aspartyl; or (d) a residue having a bulky side chain, *e.g.,* phenylalanine, is substituted for (or by) one

5   not having a side chain, *e.g.,* glycine, in this case, or (e) by increasing the number of sites for sulfation and/or glycosylation.

It is understood that one way to define the variants and derivatives of the disclosed proteins herein is to define them in terms of homology/identity to specific known sequences. Specifically disclosed are variants of anti-

10   atherogenic peptides and other proteins or peptides herein disclosed which have at least, 70% or at least 75% or at least 80% or at least 85% or at least 90% or at least 95% homology to the known sequence, such as Apo E. Those of skill in the art readily understand how to determine the homology of two proteins.

15

Polypeptide Production – Polypeptides of the invention are produced by any method known in the art. One method of producing the disclosed polypeptides is to link two or more amino acid residues, peptides or polypeptides together by protein chemistry techniques. For example, peptides

20   or polypeptides are chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert -butyloxycarbonoyl) chemistry (Applied Biosystems, Inc., Foster City, CA). A peptide or polypeptide can be synthesized and not cleaved from its synthesis resin, whereas the other fragment of a peptide or protein can be

25   synthesized and subsequently cleaved from the resin, thereby exposing a terminal group, which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, (Grant

27

GA (1992) *Synthetic Peptides: A User Guide.* W.H. Freeman and Co., N.Y.

(1992); Bodansky M and Trost B., Ed. (1993) *Principles of Peptide Synthesis.*

Springer-Verlag Inc., NY). Alternatively, the peptide or polypeptide is

independently synthesized *in vivo.* Once isolated, these independent peptides

5   or polypeptides may be linked to form a peptide or fragment thereof via

similar peptide condensation reactions.

For example, enzymatic ligation of cloned or synthetic peptide

segments allow relatively short peptide fragments to be joined to produce

larger peptide fragments, polypeptides or whole protein domains

10   (Abrahmsen L et al., *Biochemistry,* 30:4151 (1991)). Alternatively, native

chemical ligation of synthetic peptides can be utilized to synthetically

construct large peptides or polypeptides from shorter peptide fragments. This

method consists of a two-step chemical reaction (Dawson et al. *Science,*

266:776-779 (1994)). The first step is the chemoselective reaction of an

15   unprotected synthetic peptide–thioester with another unprotected peptide

segment containing an amino-terminal Cys residue to give a thioester- linked

intermediate as the initial covalent product. Without a change in the reaction

conditions, this intermediate undergoes spontaneous, rapid intramolecular

reaction to form a native peptide bond at the ligation site (Baggiolim M et al.

20   (1992) *FEBS Lett.* 307:97-101; Clark-Lewis I *et al., J.Biol.Chem.,* 269:16075 (1994);

Clark-Lewis I et al., *Biochem.,* 30:3128 (1991); Rajarathnam K et al., *Biochem.*

33:6623-30 (1994)).

Alternatively, unprotected peptide segments are chemically linked

where the bond formed between the peptide segments as a result of the

25   chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al.

Science, 256:221 (1992)). This technique has been used to synthesize analogs of

protein domains as well as large amounts of relatively pure proteins with full

biological activity (deLisle Milton RC et al., *Techniques in Protein Chemistry IV.* Academic Press, New York, pp. 257-267 (1992)).

Nucleic Acid and Vectors - The invention is also directed to an isolated nucleic acid encoding any one or more of the polypeptides disclosed herein.

5   In one embodiment, the nucleic acid comprises DNA, RNA and/or cDNA. It would be routine for one with ordinary skill in the art to make a nucleic acid that encodes the polypeptides disclosed herein since codons for each of the amino acids that make up the polypeptides are known. As non-limiting examples, the nucleic acids of the invention can be produced by recombinant,

10   *in vitro* methods, or by chemical synthetic means using machines and standard chemistry which would be known to one of skill in the art, or by *in vivo* cellular synthesis. Methods of synthesizing nucleic acids would be well know to one of skill in the art. For example, U.S. Patent No. 6,472,184 to Hegemann, issued October 29, 2002 , entitled "Method for producing nucleic

15   acid polymers" and U.S. Patent No. 6,444,111 to Montgomery, issued September 3, 2002, entitled "Electrochemical solid phase synthesis of polymers describes such synthesis" describe such synthetic methods. These references are hereby incorporated by reference in their entireties.

Additionally, the invention provides a vector comprising the nucleic

20   acid encoding any one or more of the polypeptides and peptides described herein. In certain embodiments, the invention provides a vector comprising a nucleic acid encoding at least one of the peptides of the present invention, *e.g.,* at least one of SEQ ID NOS:1-207. The vector can be a viral vector, a plasmid vector, a cosmid vector, an adenoviral vector, a phage vector, a retroviral

25   vector, an adeno-associated viral (AAV) vector, or any other vector capable of including a nucleic acid encoding a peptide or polypeptide of the invention. The vector can be an expression vector that is intended and capable of integrating into a cell genome. Other useful virus vectors include retroviruses

such as Moloney murine leukemia virus (MoMuLV); papovaviruses such as
JC, SV40, polyoma, adenoviruses; Epstein-Barr Virus (EBV); papilloma
viruses, *e.g.* bovine papilloma virus type I (BPV); vaccinia and poliovirus and
other human and animal viruses. Useful vectors and their construction are

5   disclosed in Sambrook, J. Fritsch, EF, and Maniatis, T. (1989) *Molecular
Cloning: A Laboratory Manual* (2nd ed.), Cold Spring Harbor Laboratory Press,
Plainview NY.

Host cell - The invention also provides for a host cell containing the
nucleic acid, polypeptide, peptide, and/or the vector of the invention. Such a

10   host cell is a eukaryotic cell or a prokaryotic cell. In the case of eukaryotic
cells, retrovirus or adenovirus based vectors can be used to put the nucleic
acid or the invention into the host cell. Methods known to one of skill in the
art to insert the nucleic acids or polypeptides in host cells are encompassed
within this invention. The following are non-limiting examples of such

15   methods: naked DNA transfection, lipofectin-mediated transfer,
transformation, micro-injection of nucleic acid into a cell, or calcium-
phosphate precipitation tranfection methods. Host cells can be obtained from
commercial sources such as the American Type Culture Collection (ATCC).
Host cells can be grown in liquid media culture or on tissue culture plates.

20   The growth conditions will be dependent upon the specific host cells used and
such conditions would be known to one of skill in the art. Tranfection and
growth of host cells is described in Maniatis, et al., *id.* The invention provides
for a recombinant cell expressing a nucleic acid encoding the polypeptide of
the claimed invention. The invention also provides for a recombinant cell

25   producing the polypeptide of the invention.

The vectors used in the host cells contain all or a part of a viral genome,
such as long term repeats ("LTRs"), promoters (*e.g.*, CMV promoters, SV40
promoter, RSV promoter), enhancers, and so forth. A non-limiting example of

such adenoviruses which can be employed in the present invention are well-known in the art and include more than 40 different human adenoviruses, *e.g.,* Ad12 (subgenus A), Ad3 and Ad7 (Subgenus B), Ad2 and Ad5 (Subgenus C), Ad8 (Subgenus D), Ad4 (Subgenus E), Ad40 (Subgenus F) (Wigand et al, *In:*

5   *Adenovirus DNA,* Doerfler, Ed., Martinus Nijhoff Publishing, Boston, pp. 408-441 (1986)). When the host cell is a prokaryote, bacterial viruses, or phages, can be used to deliver the nucleic acid of the invention to the host cell. A non-limiting example of such vectors are vectors based upon, *e.g.,* lambda phage. In any case, the vector may comprise elements of more than one virus. The

10   vector may additionally comprise a gene encoding a marker or reporter molecule to more easily trace expression of the vector.

        <u>Antibodies</u> – The invention also provides polyclonal and monoclonal antibodies that bind the peptides and polypeptides in the invention. For example, monoclonal antibody is also provided that

15   specifically binds to the Apo E-derived polypeptides of the invention. In some embodiments, the monoclonal antibody specifically binds to a polypeptide having an amino acid sequence of any one or more of SEQ ID NOS:1-207. One of ordinary skill in the art knows how to make or produce monoclonal antibodies, which specifically bind to a polypeptide having a

20   known amino acid sequence. (See for example, Steplewski et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:8653; Spira et al., 1984, *J Immunological Methods* 74:307, PCT Publication WO 86/01533 (1986) and U.S. Patent No. 6,458,592.) The monoclonal antibody, in some embodiments, can be chimeric *(e.g.,* U.S. Patent No. 5,843,708 to Hardman, et al., December 1, 1998, entitled "Chimeric

25   antibodies"), humanized *(e.g.,* U.S. Patent No. 6,423,511 to Nakamura, et al., issued July 23, 2002, entitled "Humanized antibodies"), primatized *(e.g.,* U.S. Patent No. 6,113,898 to Anderson, et al., issued September 5, 2000, entitled "Human B7.1-specific primatized antibodies and transfectomas expressing

said antibodies"), and/or linked to other polypeptides as fusion proteins. Portions of the monoclonal antibody can also be useful, either alone or linked to other proteins. These portions include, but are not limited to Fab (Fab')₂, Fv, etc. In another embodiment, the monoclonal antibody can be linked to a

5   carrier (*e.g.*, water, buffered water, 0.4% saline, 0.3% glycine, and the like) or can be associated with an adjuvant (*e.g.*, biliverdin, bilirubin, biotin, carnosine, chitin, etc.). Adjuvants have been used experimentally to promote a generalized increase in immunity against unknown antigens (*e.g.*, U.S. Pat. No. 4,877,611).

10   For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein (1975), *Nature*, 256:495-497, the disclosure of which is incorporated herein by reference), the trioma technique, the human B-cell hybridoma technique

15   (Kozbor et al. (1983), *Immunol. Today* 4:72, and the EBV-hybridoma technique (Cole et al. (1985), in <u>Monoclonal Antibodies and Cancer Therapy</u>, Alan R. Liss, Inc., pp. 77-96). Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778), can be adapted to produce single chain antibodies to the polypeptides of the invention. Alternatively, transgenic mice

20   may be used to express humanized antibodies to these polypeptides or fragments.

        Any of the procedures described above may be used to detect antibody binding. One such screening assay is described in "Methods for Measuring Cellulase Activities", *Meth. In Enzymol.*, 160:87-116.

25   <u>Compositions</u> - The invention provides for a composition comprising any one or more of the polypeptides, nucleic acids, vectors and/or antibodies described herein can be used to produce a composition of the invention which may also include a carrier such as a pharmaceutically acceptable carrier.

The polypeptide, nucleic acid, vector, or antibody of the invention can be in solution or in suspension (for example, incorporated into microparticles, liposomes, or cells). These compositions can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. One of skill in the art

5   knows how to make and use such targeting agents with the compositions of the invention. A targeting agent can be a vehicle such as an antibody conjugated liposomes, receptor mediated targeting of DNA through cell specific ligands, and highly specific retroviral targeting of cells *in vivo*. Any such vehicles can be part of the composition of the invention. In general,

10   receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clatrhin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways

15   serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, ligand valency, and ligand concentration.

In one embodiment, the composite comprises pharmaceutically

20   acceptable carrier. By "pharmaceutically acceptable" is meant a material or carrier that would be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. Examples of carriers include dimyristoylphosphatidyl (DMPC), phosphate buffered saline or a

25   multivesicular liposome. For example, PG:PC:Cholesterol:peptide or PC:peptide can be used as carriers in this invention. Other suitable pharmaceutically acceptable carriers and their formulations are described in Remington: *The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro,

Mack Publishing Company, Easton, PA 1995. Typically, an appropriate

amount of pharmaceutically-acceptable salt is used in the formulation to

render the formulation isotonic. Other examples of the pharmaceutically-

acceptable carrier include, but are not limited to, saline, Ringer's solution and

5   dextrose solution. The pH of the solution can be from about 5 to about 8, or

from about 7 to about 7.5. Further carriers include sustained release

preparations such as semi-permeable matrices of solid hydrophobic polymers

containing the composition, which matrices are in the form of shaped articles,

e.g., films, stents (which are implanted in vessels during an angioplasty

10   procedure), liposomes or microparticles. It will be apparent to those persons

skilled in the art that certain carriers may be more preferable depending upon,

for instance, the route of administration and concentration of composition

being administered. These most typically would be standard carriers for

administration of drugs to humans, including solutions such as sterile water,

15   saline, and buffered solutions at physiological pH.

Pharmaceutical compositions may also include carriers, thickeners,

diluents, buffers, preservatives and the like, as long as the intended activity of

the polypeptide, peptide, nucleic acid, vector of the invention is not

compromised. Pharmaceutical compositions may also include one or more

20   active ingredients (in addition to the composition of the invention) such as

antimicrobial agents, anti-inflammatory agents, anesthetics, and the like. The

pharmaceutical composition may be administered in a number of ways

depending on whether local or systemic treatment is desired, and on the area

to be treated.

25   Preparations of parenteral administration include sterile aqueous or

non-aqueous solutions, suspensions, and emulsions. Examples of non-

aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils

such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous

carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium choloride solution, Ringer's dextrose, dextrose and sodium choloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient

5   replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for optical administration may include ointments, lotions,

10 creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or

15 tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids, or binders may be desirable.

Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric

20 acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases

25 such as mon-, di-, trialkyl and aryl amines and substituted ethanolamines.

Transgenic Subjects - The invention also provides for a transgenic, non-human subject expressing a nucleic acid of the invention encoding a polypeptide or peptide of the invention. In one embodiment, the subject is an

animal or a plant. The invention also provides for a transgenic non-human subject expressing the polypeptide of the invention.

The animals can be produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Methods for producing transgenic animals would be known to one of skill in the art, e.g., U.S. Patent No. 6,201,165, to Grant, et al., issued March 13, 2001, entitled "Transgenic animal models for cardiac hypertrophy and methods of use thereof." In non-limiting embodiments, the animal is a mammal, and the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate, such as a human, monkey, ape, chimpanzee, or orangutan. The invention also provides an animal produced by the process of adding to such animal (for example, during an embryonic state) any of the cells disclosed herein.

Compositions (such as vectors) and methods are provided, which can be used for targeted gene disruption and modification to produce the polypeptides of the invention in any animal that can undergo gene disruption. Gene modification and gene disruption refer to the methods, techniques, and compositions that surround the selective removal or alteration of a gene or stretch of chromosome in an animal, such as a mammal, in a way that propagates the modification through the germ line of the mammal. In general, a cell is transformed with a vector, which is designed to homologously recombine with a region of a particular chromosome contained within the cell, as for example, described herein. This homologous recombination event can produce a chromosome which has exogenous DNA introduced, for example in frame, with the surrounding DNA. This type of protocol allows for very specific mutations, such as point mutations or the insertion of DNA to encode for a new polypeptide, to be introduced into the genome contained within the cell. Methods for performing this type of homologous recombination are known to one of skill in the art.

36

Once a genetically engineered cell is produced through the methods described above, an animal can be produced from this cell through either stem cell technology or cloning technology. For example, if the cell into which the nucleic acid was transfected was a stem cell for the organism, then this cell,

5   after transfection and culturing, can be used to produce a transgenic organism which will contain the gene modification or disruption in germ line cells, which can then in turn be used to produce another animal that possesses the gene modification or disruption in all of its cells. In other methods for production of an animal containing the gene modification or disruption in all

10   of its cells, cloning technologies can be used. These technologies are known to one of skill in the art and generally take the nucleus of the transfected cell and either through fusion or replacement fuse the transfected nucleus with an oocyte, which can then be manipulated to produce an animal. The advantage of procedures that use cloning instead of ES technology is that cells other than

15   ES cells can be transfected. For example, a fibroblast cell, which is very easy to culture and can be used as the cell in this example, which is transfected and has a gene modification or disruption event take place, and then cells derived from this cell can be used to clone a whole animal.

Disclosed are nucleic acids used to modify a gene of interest that is cloned into

20   a vector designed for example, for homologous recombination.

Methods for Making the Compositions of the Invention

The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless

5   otherwise specifically noted. For example, there are a variety of methods that can be used for making these compositions, such as synthetic chemical methods and standard molecular biology methods.

The peptide, polypeptides, nucleic acids and vectors of the invention can be used to make certain other aspects of the invention. For example, the

10   peptides and polypeptides of the invention can be used to produce the antibodies of the invention. Nucleic acids and vectors of the invention can be used to produce the peptides and polypeptides and other recombinant proteins of the invention. Host cells of the invention can be used to make nucleic acids, proteins, peptides, antibodies, and transgenic animals of the

15   invention. These synthetic methods are described above.

As described above, the polypeptides or peptides of the invention may also be used to generate antibodies, which bind specifically to the polypeptides or fragments of the polypeptides. The resulting antibodies may be used in immunoaffinity chromatography procedures to isolate or purify

20   the polypeptide or to determine whether the polypeptide is present in a biological sample. In such procedures, a protein preparation, such as an extract, or a biological sample is contacted with an antibody capable of specifically binding to one of the polypeptides of the invention, sequences substantially identical thereto, or fragments of the foregoing sequences.

25   In immunoaffinity procedures, the antibody is attached to a solid support, such as a bead or column matrix. The protein preparation is placed in contact with the antibody under conditions under which the antibody specifically binds to one of the polypeptides of the invention. After a wash to

remove non-specifically bound proteins, the specifically bound polypeptides are eluted.

The ability of proteins in a biological sample to bind to the antibody may be determined using any of a variety of procedures familiar to those

5   skilled in the art. For example, binding may be determined by labeling the antibody with a detectable label such as a fluorescent agent, an enzymatic label, or a radioisotope. Alternatively, binding of the antibody to the sample may be detected using a secondary antibody having such a detectable label thereon. Particular assays include ELISA assays, sandwich assays,

10   radioimmunoassays, and Western Blots.

The antibodies of the invention can be attached to solid supports and used to immobilize apolipoprotein E or polypeptides of the present invention. Polyclonal antibodies generated against the polypeptides of the invention can be obtained by direct injection of the polypeptides into an animal or by

15   administering the polypeptides to an animal. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies which may bind to the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from cells expressing that polypeptide.

20

Methods of Use

The invention also provides many therapeutic methods of using the nucleic acids, peptides, polypeptides, vectors, and antibodies, and compositions of the invention.

25   For example, the invention provides a method for enhancing LDL binding to a cell, the method comprising contacting, mixing or associating the cell with the polypeptide, thereby allowing the polypeptide to bind the LDL and enhance LDL binding and/or uptake with the associated cell. Also

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provided is a method for enhancing LDL and VLDL binding to a cell in a subject, the method comprising administering the polypeptide of the invention, or a composition thereof, to the subject in an amount effective to increase LDL and VLDL binding to the cell of the subject.

5   Further, the invention additionally provides a method for reducing serum cholesterol in a subject. In this method, an amount of the polypeptide of the invention, or a composition thereof, is administered to a subject in an amount effective to increase binding of LDL and/or VLDL to cells in the subject and enhance cellular uptake of serum cholesterol, thereby reducing

10   serum cholesterol in the subject.

The invention also provides a method for treating a subject with coronary artery disease or any disease or condition associated with increased serum cholesterol. In this method, an amount of the polypeptide of the invention, or a composition thereof, is administered to the subject in an

15   amount to effectively enhance cellular uptake of serum cholesterol in the subject and thereby treat the coronary artery disease or other associated disease in the subject. For example, the associated disease or condition can be dysbetalipoproteinemia, high blood pressure, atherosclerosis, angina, etc. Diseases or conditions associated with increased serum cholesterol would be

20   well known to one of ordinary skill in the art.

In addition, the invention provides for a method for reducing the risk of myocardial infarction in a subject. In this method, an amount of the polypeptide of the invention, or a composition thereof, is administered to the subject in an amount effective to increase cellular uptake of serum cholesterol

25   in the subject, to thereby treat the subject and reduce risk of myocardial infarction. The invention also provides a method for treating atherosclerosis in a subject, where an effective amount of the composition of the invention is

administered to subject to increase cellular uptake of serum cholesterol and to thereby treat the atherosclerosis in the subject.

The invention also provides for the use of the polypeptide of the invention for the making of a composition of the invention, for example, to
5   treat a disease associated with increased serum cholesterol in a subject or to reduce LDL and/or VLDL serum levels in a subject. Such a subject may be a mammal, such as a human. In another embodiment, the subject is an animal which can be a model system used to test human therapeutics. Non-limiting examples of such animals include dog, pig, primate, murine, feline, bovine, or
10   equine animals.

For delivery of the nucleic acids of the invention to a cell, either *in vitro* or *in vivo*, a number of direct delivery systems can be used. These include liposome fusion, gene gun injection, endocytosis, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids,
15   phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico- mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., *Science*, 247, 1465-1468, (1990);
20   and Wolff, J. A. *Nature*, 352, 815-818, (1991). If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation,
25   microinjection or proteoliposomes. The transduced cells can then be infused (*e.g.*, in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells

into a subject. Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and

5   cell populations by using the targeting characteristics of the carrier.

The present invention provides polypeptides, which can be delivered (administered) to a subject in many different ways. For example, the peptides or nucleic acids encoding the peptides can be delivered to a subject during an angioplasty or other medical procedure. The peptides can be expressed in a

10   plant (via transgenic technology) and the plant material can be ingested by the subject.

Infection of cells of a subject using a nucleic acid, vector, or composition of the invention can be carried out *in vitro* or *in vivo*. *In vitro* infection of cells can be carried out by adding the gene transfer vectors to the

15   cell culture medium. When infection is carried out *in vivo*, the solution containing the gene transfer vectors may be administered by a variety of modes, depending on the tissue, which is to be infected. Examples of such modes of administration include injection of gene transfer vectors into the skin, topical application onto the skin, direct application to a surface of

20   epithelium, or instillation into an organ (*e.g.,* time release patch or capsule below the skin or into a tumor). The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example *in vivo* or *in vitro*.

Thus, the compositions can comprise, liposomes, such as cationic

25   liposomes (*e.g.,* DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Furthermore, liposomes comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ

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or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, *see e.g.*, Brigham et al., *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al., *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); and U.S. Pat. No.4,897,355. Furthermore, the polypeptides or nucleic acids of the
5   invention can be administered as a component of a microcapsule that can be targeted to specific cell types, such as vessel wall cells, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

As one example, vector delivery can be via a viral system, such as a
10   retroviral vector system which can package a recombinant retroviral genome (*see, e.g.*, Pastan et al., *Proc. Natl. Acad. Sci U.S.A.* 85:4486, 1988; or Miller et al., *Mol. Cell. Biol.* 6:2895, 1986). The recombinant retrovirus is then used to infect and thereby deliver to the infected cells nucleic acid encoding the polypeptide of the invention. For example, if a nucleic acid disclosed herein is delivered to
15   the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 107 to 109 plaque forming units (pfu) per injection but can be as high as 1012 pfu per injection (Crystal, *Hum. Gene Ther.* 8:985-1001, 1997; Alvarez and Curiel, *Hum. Gene Ther.* 8:597-613, 1997). A subject can receive a single dose or injection of the composition or
20   polypeptide, or, if additional doses are necessary, they can be repeated at some time interval, such as one hour, one day, one week, one month, or other appropriate time intervals, as determined by the skilled practitioner, for an indefinite period and/or until the efficacy of the treatment has been established.

25   Nucleic acids that are delivered to cells, which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral integration systems can also be incorporated into nucleic

acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

Other general techniques for integration into the host genome include, 5 for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid 10 to be integrated into the host genome. Those of skill in the art know these systems, and the methods necessary to promote homologous recombination.

Therapeutic Uses

15   In general, when used for treatmenat, the therapeutic compositions may be administered orally, parenterally (*e.g.*, intravenously or subcutaneous administration), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, by intracavity administration, transdermally, or topically or the like, including topical intranasal administration or 20 administration by inhalant. The topical administration can be ophthalmically, vaginally, rectally, or intranasally. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the 25 nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (*e.g.*, lungs) via intubation. The exact amount of the compositions required will vary from

subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. An appropriate amount for a particular composition and a particular subject

5   can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of

10   suspension in liquid prior to injection, or as emulsions. Parenteral administration includes use of a slow release, a time release or a sustained release system such that a constant dosage is maintained.

Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the

15   skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and

20   extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counter-indications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be

25   found in the literature for appropriate dosages for given classes of pharmaceutical products. Following administration of a disclosed composition, such as a peptide, for treating, inhibiting, or preventing artherosclerosis, the efficacy of the therapeutic peptide can be assessed in

various ways well known to the skilled practitioner. For instance, one of

ordinary skill in the art will understand that a composition, such as a peptide,

disclosed herein is efficacious in treating or inhibiting artherosclerosis in a

subject by observing that the composition reduces cholesterol, LDL, or VLDL

5   levels or reduces the amount of cholesterol present in an assay, as disclosed

herein. The compositions that inhibit increased cholesterol levels, LDL levels,

VLDL levels artherosclerosis, or embolus formation as disclosed herein may

be administered prophylactically to patients or subjects who are at risk for

artherosclerosis, stroke, myocardial infarction, or embolus formation.

10   The peptides, polypeptides, nucleic acids, antibodies, vectors and

therapeutic compositions of the invention can be combined with other well-

known therapies and prophylactic vaccines already in use. The compositions

of the invention can be used in combination with drugs used to stabilize the

patient and limit damage to the heart. Such drugs include thrombolytics,

15   aspirin, anticoagulants, painkillers and tranquilizers, beta-blockers, ace-

inhibitors, nitrates, rhythm-stabilizing drugs, and diuretics. Drugs that limit

damage to the heart work only if given within a few hours of the heart attack.

Thrombolytic drugs that break up blood clots and enable oxygen-rich blood to

flow through the blocked artery increase the patient's chance of survival if

20   given as soon as possible after the heart attack. Thrombolytics given within a

few hours after a heart attack are the most effective. Injected intravenously,

these include anisoylated plasminogen streptokinase activator complex

(APSAC) or anistreplase, recombinant tissue-type plasminogen activator (r-

tPA), and streptokinase. The compositions of the invention can be combined

25   with any of these drugs. The combination of the peptides of the invention can

generate an additive or a synergistic effect with current treatments.

The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples. Rather, in view of the present disclosure that describes the current best mode for practicing the invention, many modifications and

5   variations would present themselves to those of skill in the art without departing from the scope and spirit of this invention. All changes, modifications, and variations coming within the meaning and range of equivalency of the claims are to be considered within their scope.

10   __NON-LIMITING EXAMPLES OF THE INVENTION__

__Example 1 – Single Amphipathic Helical Structure Containing Arg Residue
on Polar Face of Helix Associates with Lipid and Enhances
Lipid Uptake__

The molecular modeling shown in Figs. 1A-1F was performed to

15   determine the nature of the helical structure of the single domain peptide of the invention given changes to the residues internal to the helix. Computer software useful to carry out the molecular modeling includes programs called WHEEL, LOCATE, HELNET described in M.K. Jones, G.M. Anantharamaiah, and J.P. Segrest; "Computer programs to identify and classify amphipathic α

20   helical domains" *J. Lipid Res.* 33:287-296, 1992.

Experiments were designed to determine whether modulation of the atherogenic lipoprotein surface with positively charged residues would alter LDL binding to cell surface proteoglycans and lipoprotein uptake by the cells. Peptides were synthesized to alter residues on the atherogenic lipoprotein

25   surface by increasing the positively charged residues there. Sequence specificity is not required in the Arg-rich domain of the dual-domain peptides for enhanced uptake of atherogenic lipoproteins. This invention provides that a single amphipathic-helical structure containing at least one Arg residue on the polar face of the amphipathic helix also associates with atherogenic LDL

and VLDL and remnant lipoproteins to enhance their hepatic uptake and degradation. The positively-charged amphipathic helical peptides were tested with regard to altering heparin sulfate (HS) levels using cell culture systems.

In particular, the alteration of the residues in the helical single domain
5   peptide was carried out. Biologically active amphipathic helices have been classified into several classes (Segrest, J.P. et al. *Proteins: Structure, function and Genetics* 8:103-117 (1990)). While the class A amphipathic helix stabilizes cell membranes, class L peptides destabilize (Tytler, E.M., et al. *J. Biol. Chem.* 268:2212-2218, 1993) and lyse cells. The entire polar face of class L
10   amphipathic helix consists of Lys residues. Class L peptides possess a wide hydrophobic face and interact avidly with phospholipids, but due to their inverted wedge shape (Figs. 1A-1F), they promote hex-phase formation, *i.e.*, inverted micelle formation (or fusion process, see Figs. 12A-12F). In Figs. 1A-1F, the Lys residues are snorkeled. That is, the alkyl chains of Lys are going
15   from a hydrophobic environment to an aqueous environment with the ε-NH2 of Lys exposed to water (imagine an elephant swimming with it trying to push its trunk exposed to air and the rest of the body in water) to a maximum extent and the molecules are energy minimized using the Sybyl program. Figs. 1A and 1D show the 18L peptide; Figs. 1B and 1E show the R-18L
20   peptide; Figs. 1C and 1F show the 18A peptide. The amino acid sequence of the 18L peptide is
Ac-GIKKFLGSIWKFIWKFIKAYG-NH2 (SEQ ID NO:1), the amino acid sequence of the R-18L peptide is Ac-GIRRFLGSIWRFIRAFYG-NH2 (SEQ ID NO:2) and the amino acid sequence of the 18A peptide is Ac-
25   DWLKAFYDKVAEKLKEAF-NH2 (SEQ ID NO:3). In Figs. 12A-12F, inverted cone shaped phospholipids are shown (such as lyso PC), similar to 18A form micellar structures, and cone shaped phospholipids are shown (such as phosphatidylethanolamine, similar to 18L form inverted nonbilayer or

hexagonal phase, an intermediate to fusion process in cells. Figs 1A-1F show peptide cross sectional structures and the corresponding lipid structures (Figs. 12A-12F) can be correlated to these peptide structures. (J.P. Segrest, et al., *Structure of the plasma lipoproteins* in *"Atlas of Atherosclerosis"* Second Edition,

5   edited by P.W.F Wilson, page 56, 2000). The peptides form cross sectional shapes similar to lipids as shown in Figs. 12A-12F and thus, it is shown herein that amphipathic helical peptides with different cross sectional shapes possess different properties. For example, the dimethylation of the 18L peptide caused the peptide to become nonlytic. Similarly, the lytic activity of 18L is

10   lost by changing Lys residues to Arg and the peptide can then be used for associating with atherogenic lipoproteins and allowing enhanced uptake of atherogenic lipoproteins by the liver. As shown in Figs. 12A-12F, the tendency of phospholipids to form various types of aggregated structures is determined by the relative cross-sectional areas of the head groups versus the

15   fatty acyl chains and, to a lesser extent, the length of the fatty acyl chains. Cone-shaped phospholipids, such as lysophospholipids and many detergents (wedge-shaped in cross-section), contain a relatively large polar head group and a single fatty acyl chain and favor a positive surface curvature and the micellar phase (*i.e.*, a spheroidal particle). A lipoprotein particle is an example

20   of a micelle formed from several different lipids and proteins. Cylindric-shaped phospholipids (e.g., phosphatidylcholine) are those whose head groups and fatty acyl chains have approximately equal cross-sectional areas and favor a flat surface, the membrane bilayer phase. Inverted cone-shaped phospholipids (inverted wedge-shaped in cross-section) include

25   phosphatidylethanolamine; these contain relatively large acyl chain cross-sectional areas favoring a negative surface curvature and inverted nonbilayer phases. See Figs. 12A-12F.

Accordingly, it was deduced and understood based on these molecular

modeling experiments that a lytic peptide can be made non-lytic by increasing

the width of the polar face either by substituting Lys with Arg or by

dimethylating Lys residues. This is supported by the results shown in Fig.2

5   and described in Example 2 that follows.

Example 2 – Dimethylation of Lysine in the 18L Peptide Neutralizes the Hemolytic Property of the 18L Peptide

This example was performed to test the affect of amino acid residue

10   changes on the lytic nature of peptides of the invention. Lysine residues,

which resided on the polar face of the single domain peptide, were substituted

with arginine and such altered peptides were tested for lytic ability. Similarly,

in other peptides of the invention, lysine residues on the polar face of the

single domain peptide of the invention were dimethylated. These altered

15   peptides neutralized the hemolytic property of the 18 L peptide. A

suspension of erythrocytes (106 cells/ml) in phosphate-buffered saline with

and without peptide (10 ml of 100 mM peptide solution) was incubated for 10

min at 37°C. Hemolysis was expressed as hemoglobin content (absorbance at

540 nm) of the supernatant after centrifugation at 16,000xg for 3 min.

20   Hemoglobin content as measured in the presence of 0.1% Triton X-100 was

taken as 100% hemolysis. Results indicated that while the 18L peptide was

80% lytic, DiMe18L peptide and R-18L peptide, similar to membrane

stabilizing 18A peptide, did not cause lysis.

As shown in these experiments, the invention provides that a positively

25   charged single amphipathic helical domain associates with atherogenic

lipoproteins and enhances their hepatic uptake and degradation. However, it

is important to eliminate the cytotoxic effects of the 18L peptide. For

accomplishing this elimination of cytotoxic effects, knowing that the Arginine

50

side chain exerts increased bulkiness to the side chain of this amino acid

(similar to the dimethylation of Lys residues), an analog of the 18L peptide in

which all Lys residues were replaced by Arg was synthesized. This was the

first time that the effect of association of such peptides to atherogenic apo B-

5   containing lipoproteins was appreciated.

In sum, this example shows that addition of the 18L peptide to red cells

causes hemolysis (Fig. 2). However, dimethylation of Lys residues neutralizes

the hemolytic property that is characteristic of class L peptide (Fig. 2). While

the 18A peptide, the (DiMeK)18L peptide and the R-18L peptide do not cause

10   cell lysis, the 18L peptide caused marked hemolysis as shown in Fig. 2.

The sequence of the 18L peptide is GIKKFLGSIWKFIKAFVG (SEQ ID

NO:7) which is a Class L amphipathic helix. The sequence of the (R)18L is

GIRRFLGSIWRFIRAFYG (SEQ ID NO:8) which is a class L amphipathic helix

with the lysine residues changed to arginine. The sequence of the 18A peptide

15   is DWLKAFYDKVAEKLAEAF (SEQ ID NO:9) which is a class A amphipathic

helix.

The 18L analog peptide with all lysine residues replaced by arginine

((R)18L) is also completely devoid of any hemolytic properties (Fig. 2).

Circular dichroism (CD) resulted in the presence of lipid (DMPC) and

20   indicated that peptides 18L, R-18L and Ac-18A-NH$_2$, all possesses comparable

helicities. Model building indicated that the replacement of lysine in the 18L

peptide by arginine residues transformed the inverted wedge cross-sectional

shaped molecule into a trapezoidal shaped molecule (Figs. 1A-1F).

25   <u>**Example 3 – Effects of Analogs of the R-18L Peptide and Effects of Different**</u>
<u>**Peptide Length on Lipoprotein Uptake**</u>

These experiments were performed to test shorter analogs of the

peptides of the invention and their ability to facilitate and enhance lipoprotein

uptake by cells. In this example, two 14-residue analogs of (R) 18L (14 –

residue single domain pepetides) were designed and synthesized. The (R) 18L

peptide has the following sequence:

Ac-(R)18L-NH₂: GIRRFLGSIWRFIRAFYG (SEQ ID NO:10). One analog has

5   the four C-terminal residues deleted and has the following sequence: Ac-

(R)14L-NH₂ analog 1: GIRRFYGSIWRFIR (SEQ ID NO:11). Another analog

has two residues each deleted from the N-terminal and the C-terminal as

follows: Ac-(R)14L-NH₂ analog 2: --RRFYGSIWRFIRAF (SEQ ID NO:12). As

shown in Figs. 8A-8B, the R-14L-2 peptide but not the R-14-L-1 peptide

10   enhanced the uptake of LDL *in vitro*. The peptide R-14L-2 but not R-14L-1

was also effective in decreasing plasma cholesterol in Apo E null mice. It is

important to understand the differences between the behavior of these two

peptides with respect to their secondary structure, lipid affinity and ability to

associate with atherogenic lipoprotein particles. These analogs enable the

15   determination of the mechanism by which the atherogenic lipoproteins are

taken up by HepG2 cells. From these results, it was clear that positively

charged Arg-containing peptides that are helical and are able to associate with

atherogenic lipoproteins, are also able to enhance the uptake of atherogenic

lipoproteins to result in decreased levels of plasma cholesterol. Thus, several

20   variations of such a structure are possible.

In Fig. 9, the effect of R14L peptides on uptake of human LDL in

Chinese hamster ovary (CHO) cells is shown. CHO cells were selected

because of the availability of various mutants with changes in proteoglycan

expression to study the role of proteoglycans in the uptake of atherogenic

25   lipoproteins. Similar to these *in vitro* results, the peptide R-14L-2 is able to

enhance atherogenic lipoprotein uptake and degradation in apo E null mice.

The R18L peptide was sparingly soluble in aqueous solvents. Analogs have

been synthesized wherein Ile residues in all of these peptides have been

replaced by Leu. The resulting peptide analogs have been found to possess increased solubility. These new analogs can be effective in enhancing hepatic uptake of atherogenic lipoproteins.

The Ac-(R)18L-NH₂ peptide was effective in reducing plasma

5   cholesterol in several dyslipidemic mouse models in a dose-dependent manner. The 14-residue analogs were designed to further determine minimal structural motifs required for this reduction in plasma cholesterol, and to improve solubility at a neutral pH.

Fig. 10 shows the effects on cholesterol levels of peptides Ac-(R)14L-

10   NH₂ analog 1, which is a peptide derived from the original sequence (in this case R18L) by the deletion of a part of the original sequence or substitution of existing amino acids with others or derivatizing certain functional amino acids in the sequence such as dimethylation of Lys residues, (at 100 μg/mouse; n=5) and Ac-(R)18L-NH₂ (at 50 μg/mouse; n=5), administered intravenously into

15   fasted apo E null mice. The peptides were dissolved in PBS pH=5.0. Control mice (n=5) received PBS pH=5.0. Data shown represent mean ± SEM.

As shown in Fig. 10, peptide Ac-(R)14L-NH₂ analog 1 was not effective in reducing plasma cholesterol in fasting apo E null mice when injected as free peptide at a dose of 100 μg/mouse at pH=5. Peptide Ac-(R)18L-NH₂ was

20   effective in reducing plasma cholesterol in these animals at a dose of 50 μg/mouse. Peptide Ac-(R)14L-NH₂ analog 2 was freely soluble at pH=5.0, but at pH=7.4 was only partially soluble, with a light suspension apparent. Addition of DMPC at a 1:1 ratio (w/w) completely clarified this suspension.

Effects on cholesterol levels of peptide Ac-(R)14L-NH₂ analog 2 (at 100

25   μg/mouse), administered intravenously into fasted apo E null mice as free peptide or as peptide:DMPC complexes at two pH levels. n=5 in all cases is shown in Fig. 11. Peptides were dissolved in PBS at the pH shown. Data shown represent mean ± SEM. As shown in Fig. 11, injection of the free

peptide at pH=7.4 reduced plasma cholesterol in fasting apo E null mice (100

μg/mouse, i.v.) to a greater extent than did injection of peptide:DMPC

complexes at either pH=5.0 or 7.4. Peptide:DMPC complexes at either pH

were essentially equivalent in their ability to reduce plasma cholesterol. These

5   results indicate that peptide:lipid complexes are also able to enhance the

uptake of atherogenic lipoproteins.

10

Example 4 – *In vitro* Assays Show R-18L Peptide Enhances Uptake of LDL and VLDL by HepG2 Cells

These experiments were performed to test the bioactivity of the R-18L

peptide in cell culture. *In vitro* studies showed that R-18L was effective in

15   enhancing the uptake of LDL and VLDL by HepG2 cells (Figs. 3A-3B).

Specifically, ^{125}I-LDL and ^{125}I-VLDL and the R-18L peptide were incubated

with HepG2 cells *in vitro* for 2 hours to determine the cellular uptake of the

iodinated compounds. The cell-associated counts representing LDL and

VLDL showed the uptake of LDL and VLDL by the cells. H/H represent

20   uptake of the lipoproteins in cells treated with heparinase/heparitinase. While

in the case of VLDL, the enhanced uptake was completely abrogated in the

presence of H/H, there was a significant enhancement of uptake in the case of

LDL. These results suggest that LDL is taken up by more than one pathway

in the presence of these peptides. In case of VLDL, the peptide-mediated

25   uptake appeared to be predominantly via an HSPG pathway. A peptide of

the invention containing all-arginine residues in place of arginine and lysine

residues has been shown to be the most active in HepG2 cells in enhancing the

uptake and degradation of atherogenic lipoproteins. Examples of atherogenic

lipoproteins are VLDL and LDL. These studies show that the receptor
binding domain, LRKLRKRLLR (SEQ ID NO:6) (141-150 residues from Apo E)
of Apo E enhances the uptake of atherogenic lipoproteins.

5   **Example 5 – _In vivo_ Studies Show Single Domain R-18L Peptide Lowers
    Plasma Cholesterol In Apo E(-) Mice More Effectively Than a
    Dual Domain Peptide.**

These studies were carried out to assess the bioactivity of the single
domain R-18L peptides _in vivo_ and compare its activity with the known
10   activity of a dual domain Apo-E derived peptide. In this case, the bioactivity
refers to the activity of lowering serum cholesterol in a subject (_e.g._, in a
transgenic mouse). In this example, the 18L peptide analogs were
administered to dyslipidemic mice. Two mouse models were selected: the
first was C57BL6 mice on high fat diet and the second was apo E(-) mice,
15   which possess severe dyslipidemia (400 mg/dl to 600 mg/dl plasma cholesterol
and develop atherosclerosis spontaneously even on a normal chow diet).
While the peptide 18L was highly toxic to mice (four out of six mice injected
with 18L 100 µg/mouse died), the R-18L single domain peptide was able to
lower plasma cholesterol in apo E(-) mice more effectively than the dual
20   domain peptide (Figs. 5 and 6). The mice administered with R18L (100
µg/mouse) were completely normal, even when 10 times excess of 18L (lethal
dosage) peptide was administered intravenously.

Using mutant cells lacking proteoglycans, results show that heparan
sulphate proteoglycans (HSPG) and other receptors play a role in the uptake
25   of dual-domain peptide-VLDL complexes. Peptide Ac-(R)18L-NH$_2$ was found
to have poor solubility at neutral pH and addition of DMPC did not improve
this solubility. However, Ac-(R)18L-NH$_2$:DMPC (1:1 w/w) complexes were
found to be very soluble in PBS at pH=5. Thus, all experiments used PBS at
pH=5 as controls.

Figure 4 shows the level of plasma cholesterol 2 min and 5 hours after injection of apo E null mice with peptide Ac-R18L-NH₂ intravenously at a dose of 100 μg/mouse. Blood samples were taken immediately before injection and at 2 minutes and at 5 hours after injection and measured for

5   cholesterol. A control of only PBS was also measured. Total cholesterol in the blood samples was measured, and is expressed in Fig. 4 as mean ± SEM. When the peptide was injected into apo E null mice, plasma cholesterol was reduced by approximately 90% within two minutes and that reduction was maintained for at least five hours (Fig. 4). However, the reduction in plasma

10   cholesterol was in the lipoproteins, including HDL. Dose dependency of cholesterol reduction in apo E null mice showed that a substantial reduction occurred at a dose of 50 μg of peptide per mouse (Fig. 5), and that reduction was limited to the VLDL and IDL/LDL fractions. Fig. 5 shows dose dependency of cholesterol reduction in apo E null mice and shows reduction

15   at the dose of 50 μg/mouse. Feces were collected for 24 hours following injection of 100 μg of Ac-(R)18L-NH₂:DMPC complexes (n=2) or PBS (n=5). Fecal sterols were extracted, and total cholesterol was determined manually (Sigma Infinity). As shown in Fig. 6, total fecal cholesterol from peptide-injected animals was nearly twice that of PBS-injected controls, suggesting

20   increased hepatic secretion of cholesterol (and, presumably, bile salts) into the gut. Total feces were collected from individually-caged mice for 24 hours following intravenous injection of Ac-(R)18L-NH₂ into apo E null mice at a dose of 100 μg/mouse. Total 24-hour fecal cholesterol was then calculated. Feces from each mouse were weighed, lyophilized, and then re-weighed. 100

25   mg dried feces was then homogenized, and total lipids were extracted. The dried extract was resuspended in isopropanol, and cholesterol was measured manually. Data shown in Fig. 6 are mean ± SEM for control and mean ± range for peptide.

In contrast to Ac-hE18A-NH₂, [¹²⁵I]Ac-(R)18L-NH₂:DMPC complexes were able to associate with LDL from LDL-R null mice (Fig. 7). Peptide [¹²⁵I]Ac-(R)18L-NH₂ was mixed with plasma from an LDL-R null mouse at a proportion of 100 μg peptide:1 mL plasma. The plasma lipoprotein profile

5   was determined using the CLiP method (Garber and Anantharamaiah, *J. Lipid. Res.*, 2000), and fractions were collected for measurement of radioactivity. Thus, peptide:lipid complexes are good candidates for administration that would result in enhanced uptake of atherogenic lipoproteins. The effect of the single domain peptide of the invention should

10 last longer than any dual domain peptide since lipid-associated peptide is helical and the backbone is not accessible for proteolytic cleavage (degradation). Thus, several slow-release formulations of peptides with lipids are tested to determine the optimal formulation for delivery.

Since binding of the peptide of the invention to a lipoprotein surface is

15 the main requirement for the enhanced uptake of atherogenic lipoproteins, shorter peptides derived from 18L were designed, synthesized and tested. For this purpose, two peptides were derived from R-18L. This is shown in Fig. 8A (peptide R14 L-1) and Fig. 8B (peptide R14-L2). In the linear sequence of R-18L:GIRRFLGSIWRFIRAFVG (SEQ ID NO:4) two amino acids on the N- and

20 C-termini are removed (R-14L-1, Fig. 8A) and four amino acids from N-terminus of R18L are deleted (in R-14L-2, Fig. 8B). Shortening of the peptide may have an effect on the secondary structure and lipid affinity. However, a Y and a W were maintained in both the analogs to enable fluorescence studies and radiolabeling of the peptide analogs.

25

Example 6 – Making Compositions and Formulations of the Polypeptide of the Invention

A. Synthesis of Peptides of the Invention

Peptide synthesis is a routine method that would be well known by one of ordinary skill in the art. There are service companies that exist which provide the service of making peptides given a desired amino acid sequence. In a non-limiting example, peptides are synthesized by the solid phase

5   method using automatic peptide synthesizer using the Fmoc chemistry. Typically, rink amide resin is used for the synthesis. Fmoc amino acids are added using HBTU and FMOC at each stage of the synthesis and is removed by treating the blocked products with piperidine. After the addition of amino acids, the N-terminal amino group is acetylated using acetic anhydride. The

10   peptide resin is treated with 70% triflouroacetic acid in dichloromethane (in presence of 1% anisole, 1% metcaptoethanol and 1% water). After the evaporation of the solvent, ether is added and the precipitate washed with additional amounts of ether. The peptide is purified by using HPLC (C-4 Michell Muller column) 35-60% acetonitrile (in the presence of 0.1%

15   trifluoroacetic acid) in 5 hrs with a flow rate of 4 ml/min. The purity of the peptide was determined by analytical HPLC and mass spectral analyses.

B. Preparation of Peptide Lipid Complexes

The peptide of the invention easily associates with

20   dimyristoylphosphatidyl choline (DMPC), egg PC, palmitoyloleylphosphatidyl choline (POPC). Peptide and lipid mixtures (1:5 by weight ratio) are prepared by spontaneous mixing. A clear solution is obtained upon mixing of peptide with lipid. Whether the peptide:lipid complex is clear or not, the mixture is sterilized by membrane filtration and

25   used for experiments.

C. Peptide:Lipid Slow Release Formulations

The following description provides how to make peptide:lipid slow
release formulations. The slow release lipid formulations of peptide using
lipid mixtures can be prepared using the following general procedure
(Ramprasad, M.P. et al., *J. Controlled Release* 79:207-218, 2002). Specifically, in a

5   non-limiting example, 6 ml of an aqueous solution of the peptide (2 mg/ml
containing 25 mM acetic acid, pH 4.0 and 4% sucrose) is emulsified with 6 ml
of chloroform solution containing phospholipids (13.2 mM
dipalmitoylphosphatidyl choline (DPPC), 13.2 mM dioleylphosphatidyl
choline (DOPC) and 5.6 mM dipalmitoylphosphatidyl glycerol (DPPG)

10   cholesterol (39.8 mM), and triglycerides (2.44 mM triolin and 2.44 mM
tricaprylin). This first emulsion is mixed with the second aqueous solution
containing 3.2% glucose and 40 mM lysine to form the water-in-oil-water
(second) emulsion. Chloroform is removed by flushing nitrogen over the
surface of the mixture at 37 °C. The resulting emulsion MLV particles are

15   harvested by centrifugation for 10 min at 600 x g, washed, and resuspended in
isotonic buffer. Such a procedure has been used for the dual-domain peptide
slow release when administered in dyslipidemic mice (*Id.*).

20   ## Example 7 - Binding, Internalization and Degradation of Atherogenic Lipoproteins in Cultured Cells

This experiment was carried out to follow the events subsequent to the
binding of atherogenic lipoproteins to cultured cells. HepG2 cells (obtained
from American Type Culture Collection (ATCC), Manassas, VA) are used for

25   determining peptide-mediated enhanced uptake of LDL or VLDL. Cells are
grown in DMEM in six-well plates and used at 75% to 90% confluence. The
seeding density of the cells that is used is $1.5\text{-}3.0 \times 10^5$ cells/ml. Cells are
treated with DMEM containing lipoprotein-deficient serum (LPDS) 24 h prior
to use to upregulate LDL incubated with the indicated concentration (0 to 50

μg of ^{125}I-LDL) at 4°C for 2 h in presence or absence of peptides. Nonspecific

binding is assessed in the presence of 50-fold excess of unlabelled LDL with or

without peptides. After washing with ice-cold PBS, (containing 2 mg/ml BSA)

to remove excess labeled lipoproteins, cells are incubated with excess dextran

5   sulfate (4 mg/ml Pharmacia, M_r of 500,000) or heparin (Sigma Chemical Co.,

St. Louis, MO, 10 mg/ml) for 1 h to release specifically bound ^{125}I-LDL. The

counts in the dextran sulfate wash reflect the amount of LDL bound to cells.

Cells are then washed with PBS and then dissolved in 0.1 N NaOH and a 0.5

ml aliquot of the cells suspension is counted. The counts reflect the amount of

10   LDL internalized. The amount of protein is estimated using the Lowry

method. Degradation of LDL is assessed by precipitating ^{125}I-LDL using 17%

trifluoroacetic acid and incubating at 4 °C for 30 min. The precipitate is

removed by centrifugation. The supernatant is treated with 10 μl of 40% KI

and 40 μl of 10% hydrogen peroxide. The free ^{125}I is extracted with 2 ml

15   chloroform. The upper aqueous layer is then counted for radioactivity. These

counts represent the amount of monoiodi-tyrosine produced by the

degradation of apo B in lipoproteins. Cell surface HSPG is removed by

treating cells with heparinase and heparitinase (Sigma Chemical Co., St. Louis,

MO) at a concentration of 3 u/ml of medium for 2 h at 37 °C. In all cell

20   experiments an average value of triplicate experiments are considered.

Counts are detected inside the cultured cells and it is shown that LDL and

VLDL are bound, internalized and degraded in the cultured cells in the

presence of the peptide of the invention.

25   **Example 8 - *In vivo* Studies**

This experiment is carried out to show the usefulness of the peptides of

the invention *in vivo* for lowering serum cholesterol. Peptide or peptide-lipid

complexes were administered intravenously to mice. After some time period

(as specifically shown in various figures), plasma samples were obtained by performing a retro-orbital bleed of the animals. The samples were analyzed by the column lipoprotein profile as previously described (Garber, D. et al. *J. Lipid Res.* 2000, 41:1020-1026). Briefly, 5 to 10 μl of the plasma was analyzed

5　using a single superose (Pharmacia, Piscataway, NJ) column. Immediately following the column run, cholesterol reagent is introduced through a mixing tee, and the eluent:reagent mixture enters a post-column reaction coil. Cholesterol content of the reaction mixture is determined at 500 nm spectrophotometrically and the data points are collected into a computer. The

10　resulting profiles are decomposed into component peaks and analyzed for relative area using PeakFit® computer software (SPSS Science, Chicago, Il). Absolute cholesterol value for total cholesterol is determined by comparisons with control samples of known values. The results would show a decrease in serum cholesterol in the subjects that received the polypeptides of the

15　invention.

EQUIVALENTS

　　Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific

20　embodiments described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

What is claimed is:

1.   A synthetic apolipoprotein-E mimicking polypeptide comprising an amino acid sequence selected from the group of

(i) X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y-Y-X or the reverse sequence thereof,

(ii) Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y or the reverse sequence thereof,

(iii) Y-Y-X-X-Y-Y-Arg-Y-Y-Arg-X-Y-Y-X or the reverse sequence thereof, and

(iv) X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg or the reverse sequence thereof,

wherein X is glycine, threonine, serine or alanine,

wherein Y is a hydrophobic amino acid,

wherein the polypeptide comprises an acetyl group at the N-terminus and an amide group at the C-terminus, and

wherein the polypeptide consists of a single domain.

2.   The polypeptide of claim 1, wherein Y is selected from the group consisting of phenylalanine, tyrosine, leucine, isoleucine, valine, and tryptophan.

3.   The polypeptide of claim 1, wherein the polypeptide comprises from about 10 amino acids to about 30 amino acids in length.

4.   The polypeptide of claim 1, wherein the polypeptide comprises a sequence of consecutive amino acids selected from the group of SEQ ID NOS:1-207.

5.   The polypeptide of claim 1, wherein the polypeptide comprises the sequence Gly-Ile-Arg-Arg-Phe-Leu-Gly-Ser-Ile-Trp-Arg-Phe-Ile-Arg-Ala-Phe-Tyr-Gly (SEQ ID NO:5).

6.   The polypeptide of claim 1, which is a recombinant polypeptide.

7.   The polypeptide of claim 1, which is a synthetic polypeptide.

8.   The polypeptide of claim 1, which is a peptidomimetic.

9.   An isolated nucleic acid encoding the polypeptide of claim 1.

10.   The nucleic acid of claim 9, wherein the nucleic acid comprises DNA, RNA and/or cDNA.

11.   A vector comprising the nucleic acid of claim 9.

12.   A host cell comprising the nucleic acid of claim 9.

13.   The host cell of claim 12, which is eukaryotic or prokaryotic.

14.   The polypeptide of claim 1, wherein the polypeptide enhances binding of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) to a cell.

15.   The polypeptide of claim 1, wherein the polypeptide enhances degradation of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) by a cell.

16.   A composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

17.   The composition of claim 16, wherein the carrier comprises dimyristoylphosphatidyl (DMPC), phosphate buffered saline or a multivesicular liposome.

18. A monoclonal antibody that specifically binds to the polypeptide of claim 1.

19. A method for enhancing LDL binding to a cell, the method comprising contacting the cell with the polypeptide of claim 1.

20. A method for enhancing LDL and VLDL binding to a cell in a subject, the method comprising administering the polypeptides of claim 1, or a composition thereof, to the subject in an amount effective to increase LDL and VLDL binding to the cell of the subject.

21. A method for reducing serum cholesterol in a subject, the method comprising the step of administering to the subject an amount of the polypeptides of claim 1, or a composition thereof, effective to increase binding of LDL and/or VLDL to cells in the subject, thereby reducing serum cholesterol in the subject.

22. A method for treating a subject with coronary artery disease, the method comprising the step of administering to the subject an amount of the polypeptides of claim 1, or a composition thereof, to thereby treat the subject.

23. A method for treating a subject with dysbetalipoproteinemia, the method comprising the step of administering to the subject an amount of the polypeptide of claim 1, or a composition thereof, to thereby treat the subject.

24. A method for reducing the risk of myocardial infarction in a subject, the method comprising the step of administering to the subject an amount of the polypeptide of claim 1, or a composition thereof, to thereby treat the subject.

25. A method for treating atherosclerosis in a subject, the method comprising the step of administering to the subject the polypeptide of claim 1, or a composition thereof.

26. A recombinant cell comprising the nucleic acid of claim 9.

27. A recombinant cell producing the polypeptide of claim 1.

28. A transgenic, non-human subject comprising the nucleic acid of claim 9.

29. The transgenic subject of claim 28, wherein the subject is an animal or a plant.

30. A transgenic non-human subject expressing the polypeptide of claim 1.

31. The method of claim 19, wherein the administration is oral, parenteral, by intramuscular injection, by intraperitoneal injection, transdermal, extracorporeal, topical, intranasal or by inhalant.

32. The method of claim 19, wherein the subject is a human subject.

33. The method of claim 19, wherein the subject is mammal is a mouse, a rat, a rabbit, a cow, a sheep, a pig, or a primate.

34. The method of claim 33, wherein the primate is a human, a monkey, an ape, a chimpanzee, or an orangutan.

Figs. 1A-1F

FIG. 2

FIG. 3A

FIG. 3B

Ac-R18L-NH$_2$ i.v. (100 µg/mouse)
Apo E null, fasted

FIG. 4

FIG. 5

24 h fecal total cholesterol
(collected from time of injection;
apo E null; 100 µg R18L i.v.)
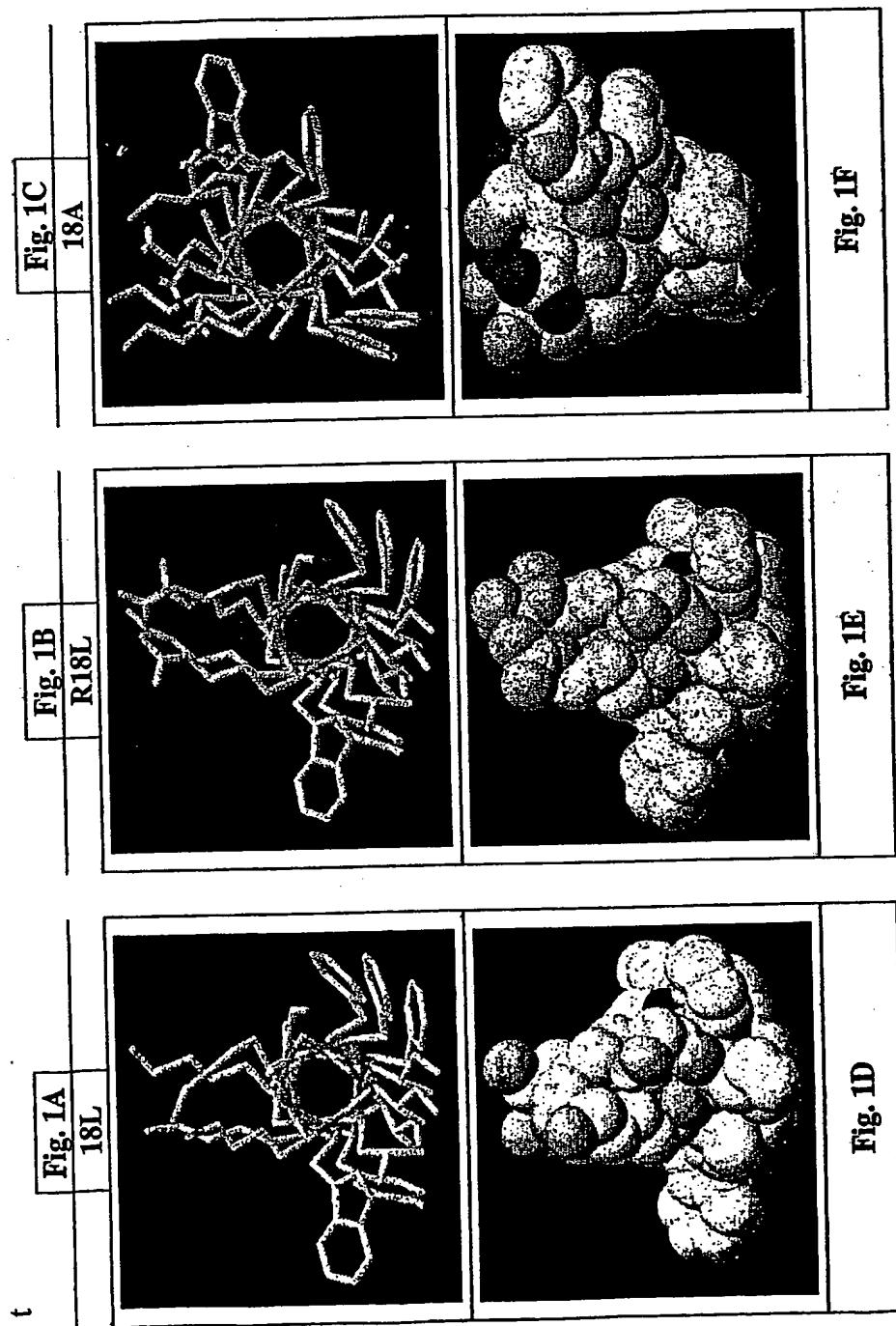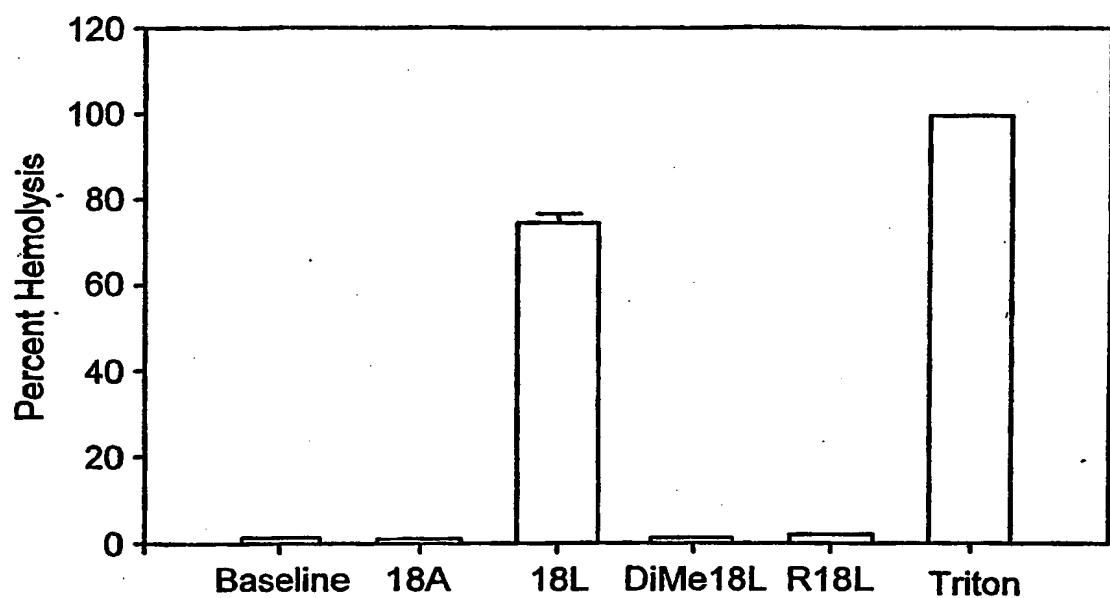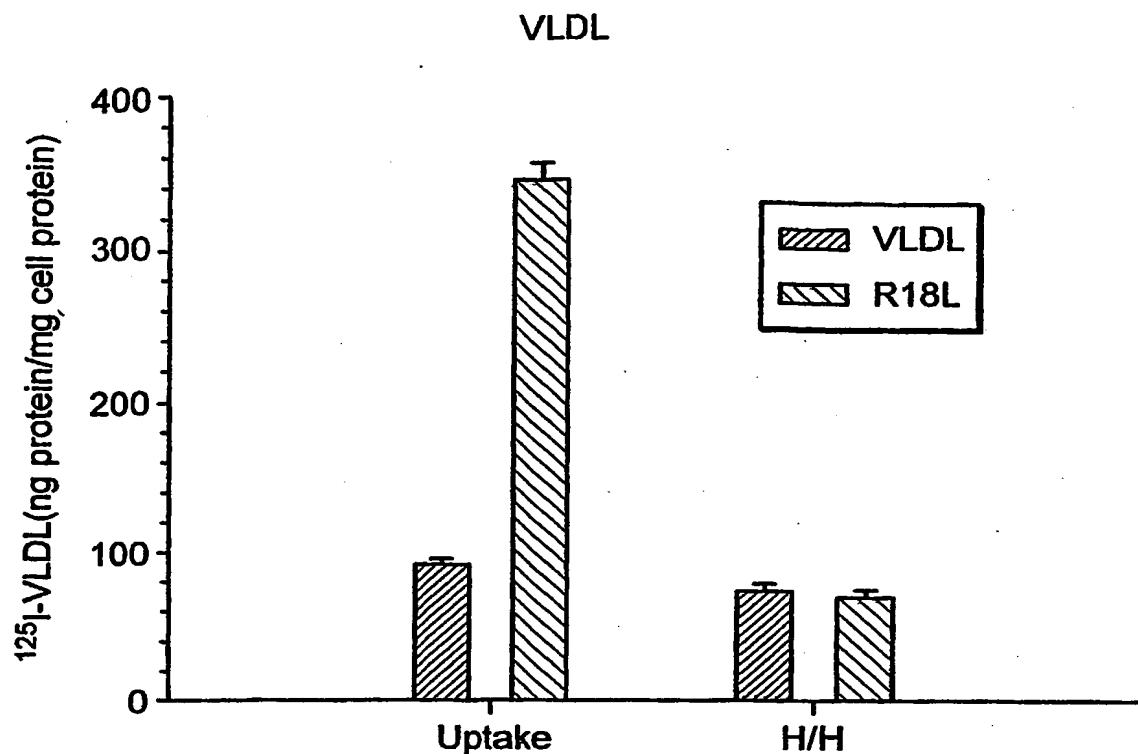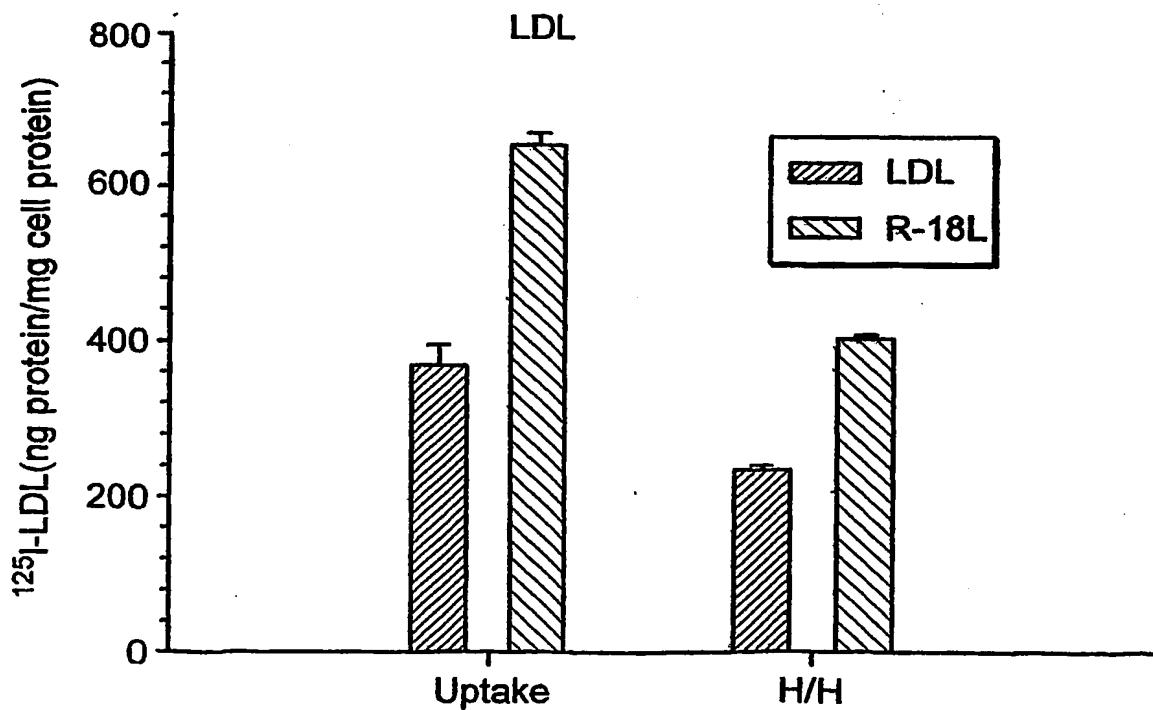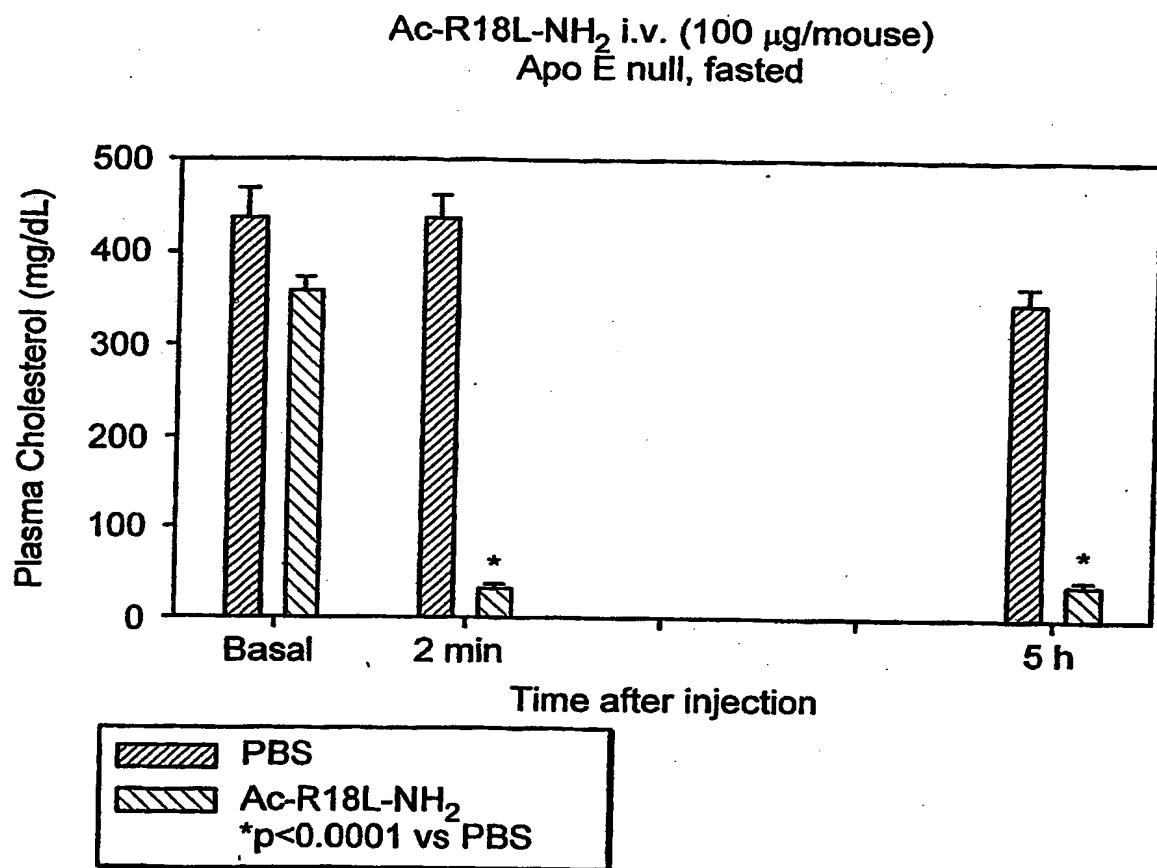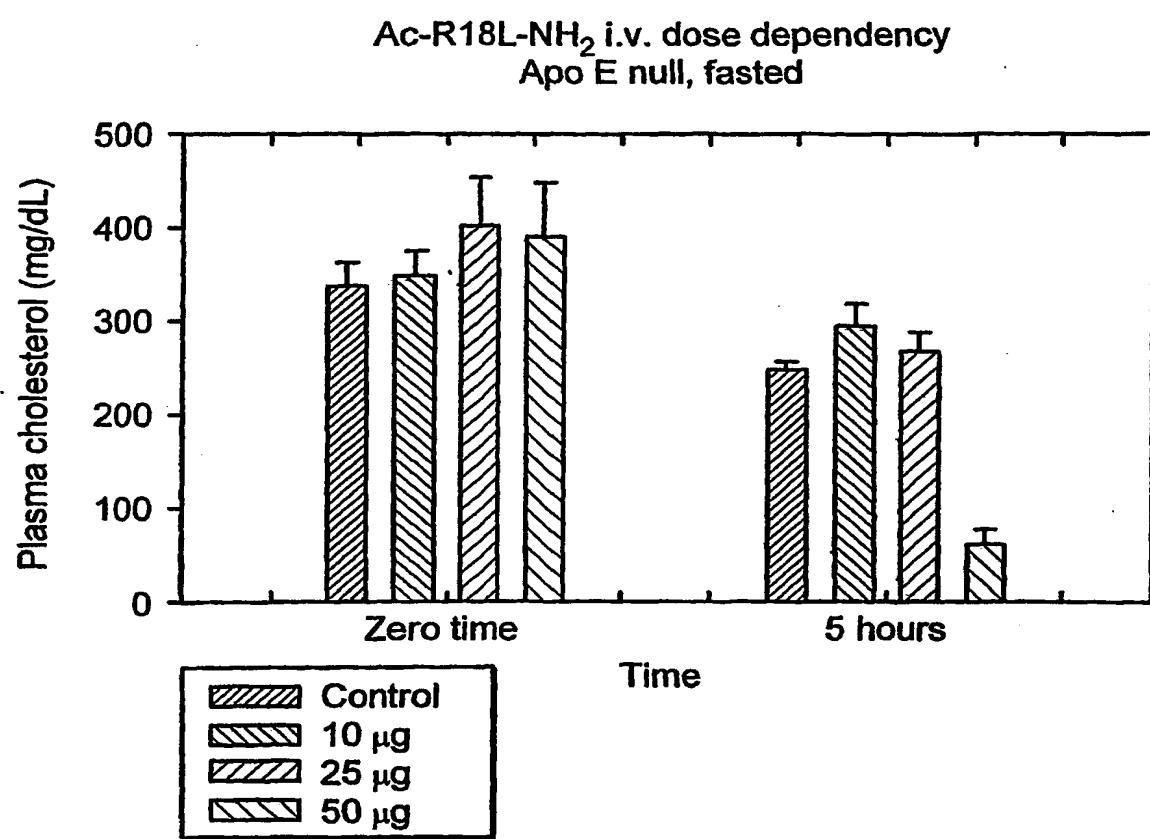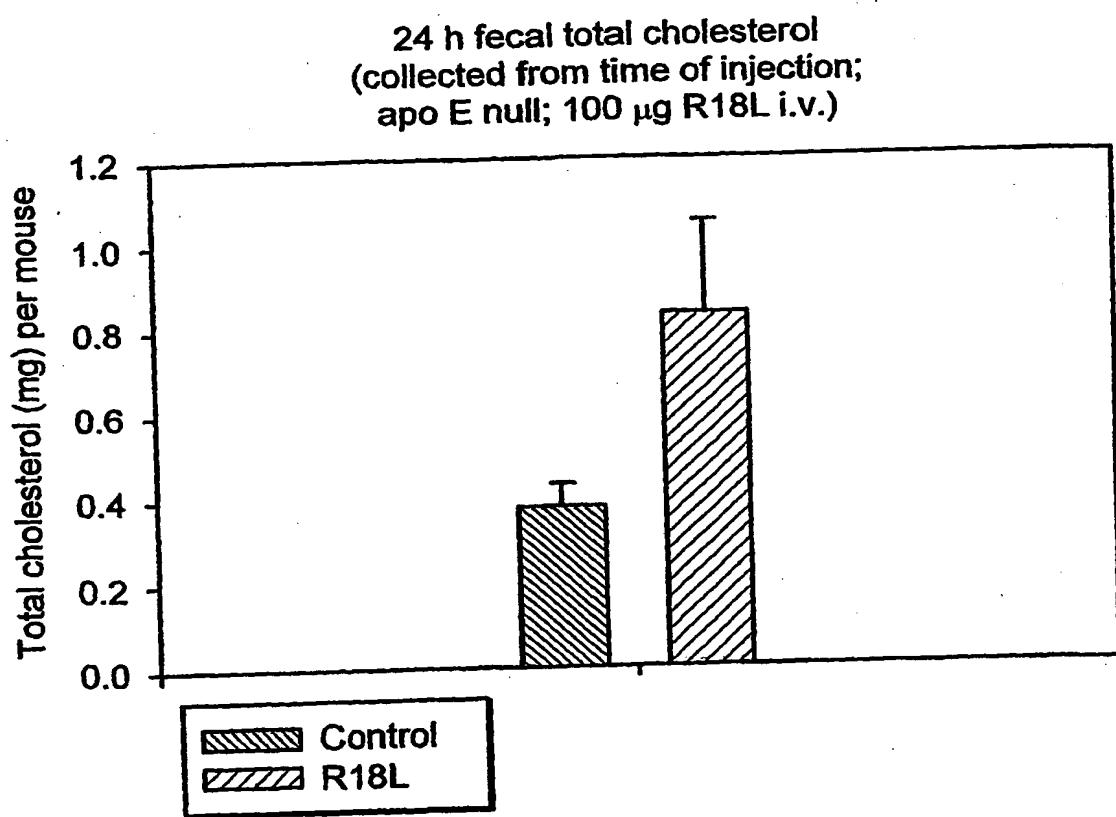
FIG. 6

In vitro mixing experiment
[125I]Ac-(R)18L-NH2
LDLR-/-, normal chow, fasted
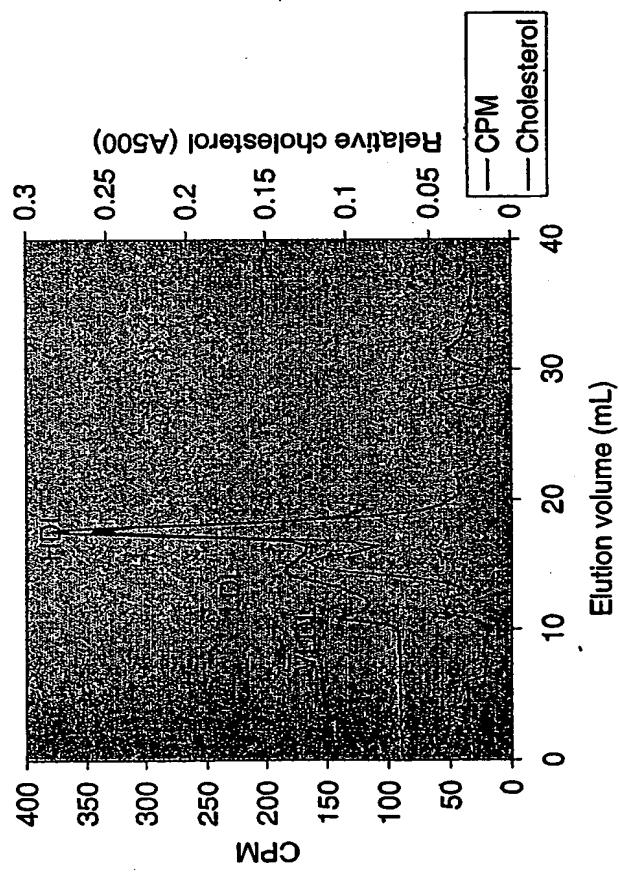
Fig. 7

R14L-1

FIG. 8A

R14L-2

FIG. 8B

FIG. 9

Apo E null; i.v. injections of two
single domain cationic peptides
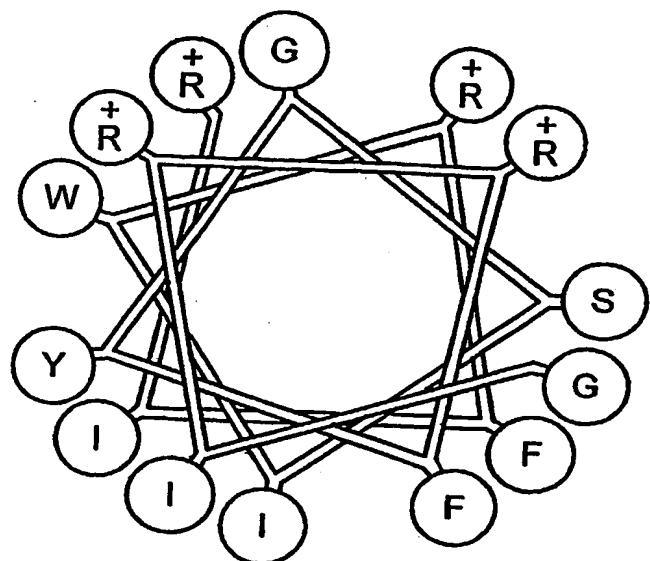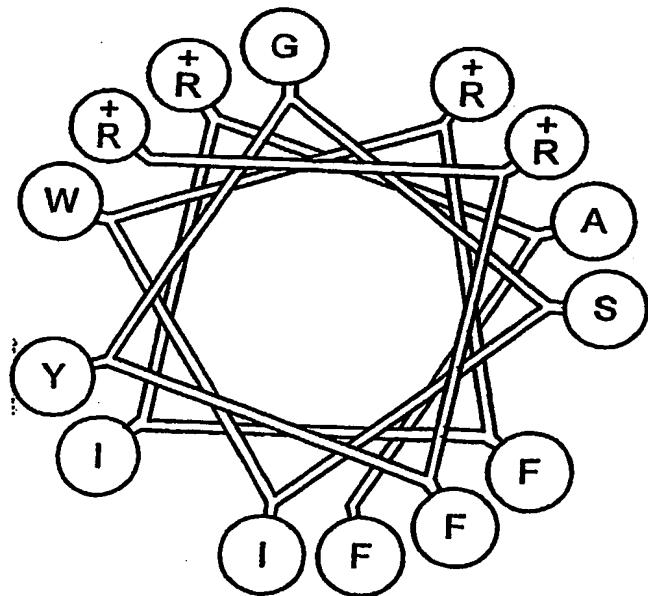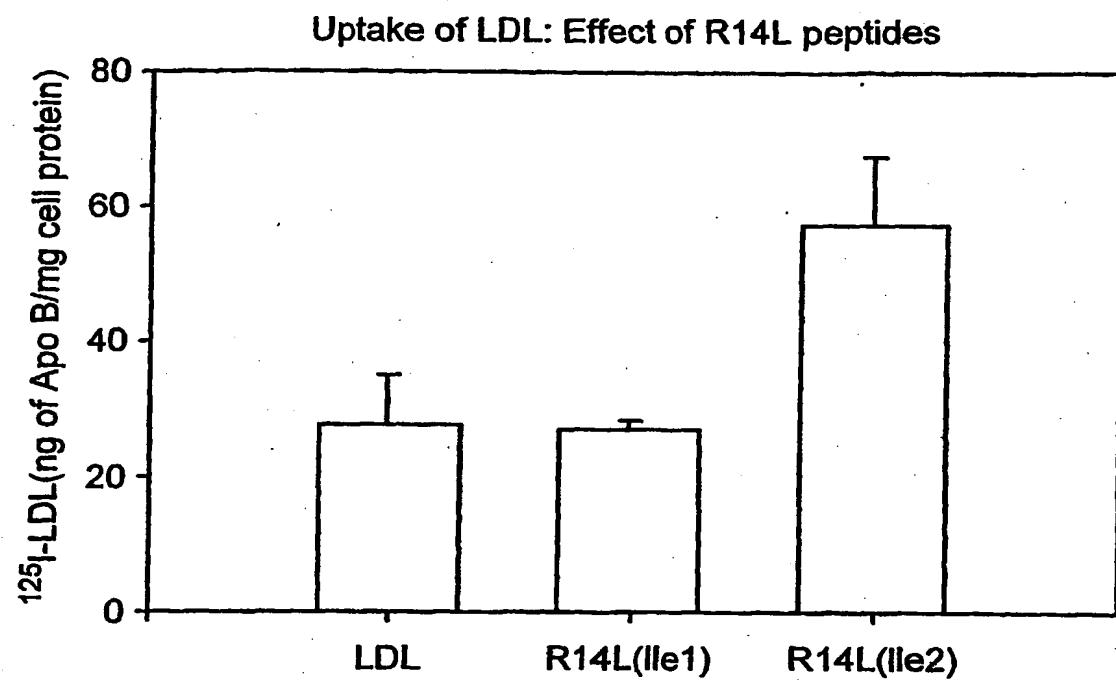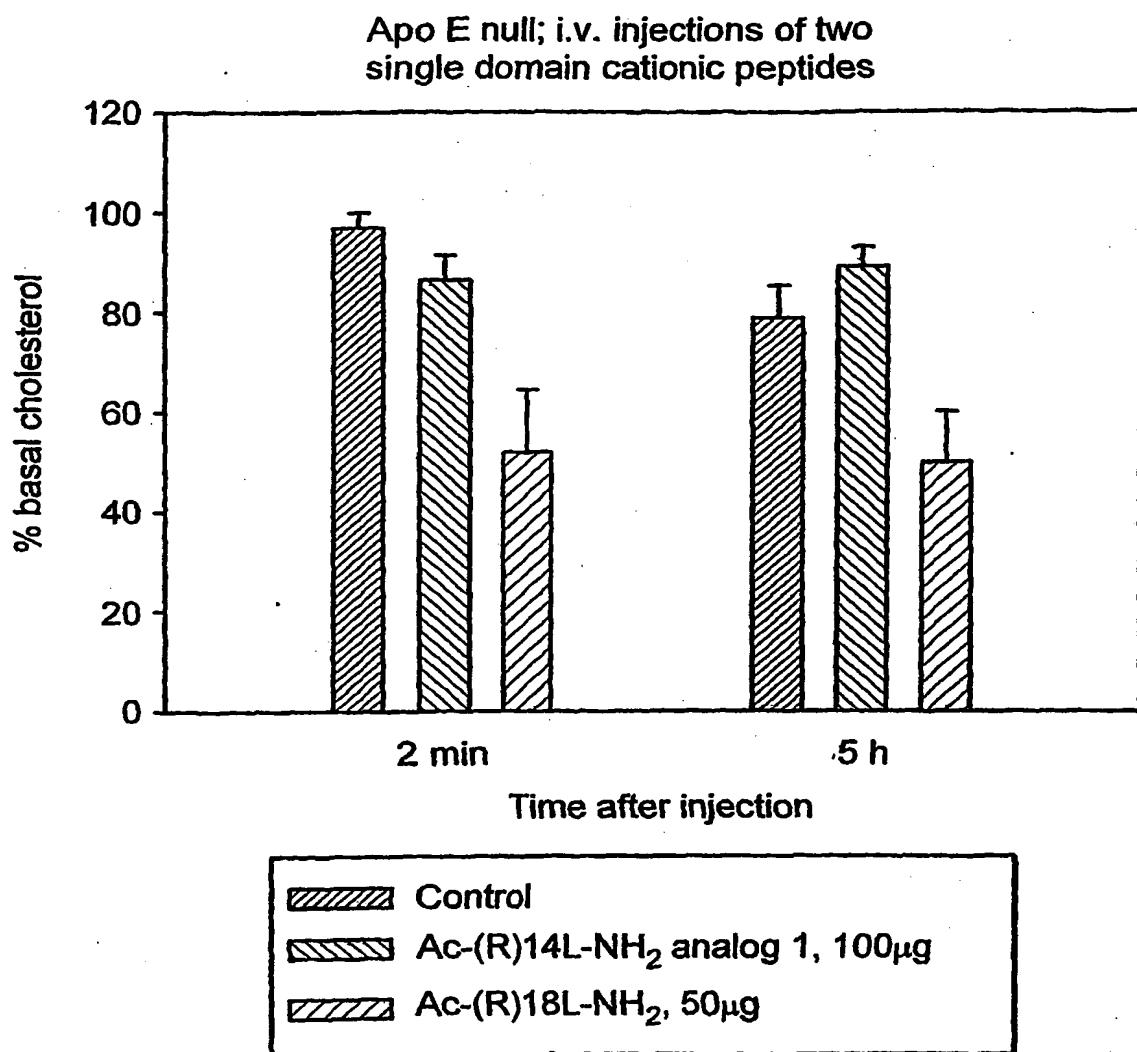
FIG. 10
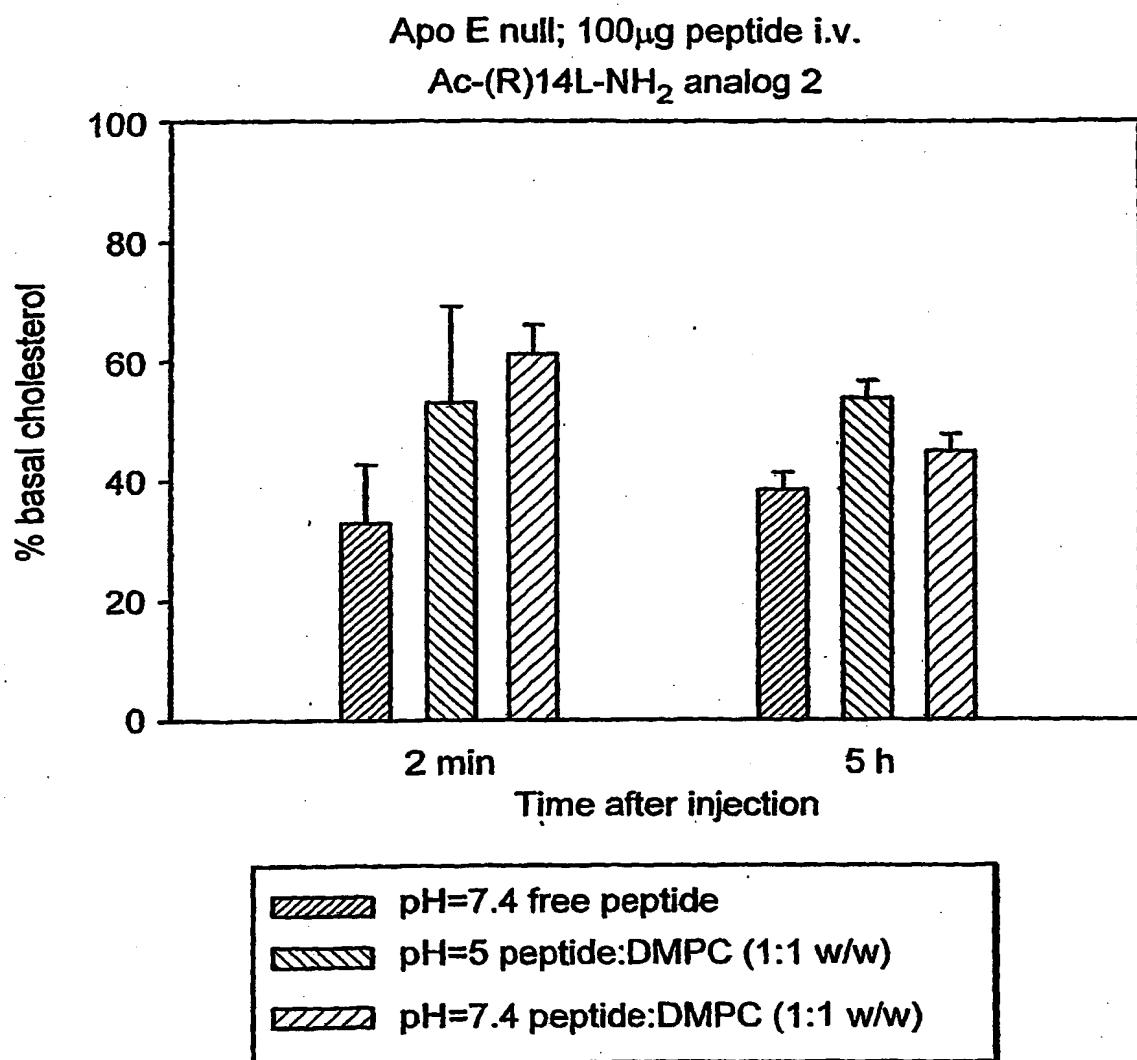
FIG. 11
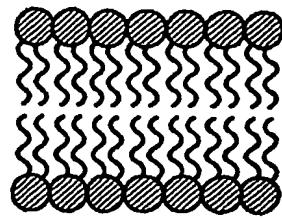
Bilayer
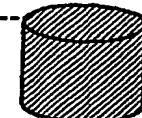
FIG. 12A

Cylindric

FIG. 12B
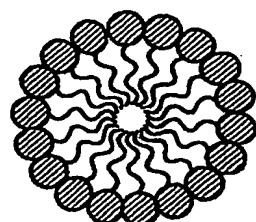
Micellar

# FIG. 12C
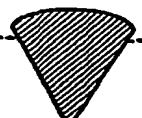
Inverted cone

FIG. 12D

Hexagonal (H$_{II}$)

FIG. 12E

Cone

FIG. 12F